

ORIGINAL

Dexmedetomidine attenuates surgery-induced cognitive deficit and hippocampal *Mapt* expression in aged mice

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ABSTRACT

Background

Postoperative cognitive dysfunction is a significant complication, but the mechanisms underlying this condition and its impact on the brain network are not fully elucidated. The goals of this study were to verify how the transcriptional network in the hippocampus of aged mice changes following sevoflurane/surgery-induced stress and to substantiate how dexmedetomidine influences the cognitive function and mRNA changes in the hippocampus.

Materials and methods

We first performed transcriptome analysis to confirm the changes of mRNA expression in the Naïve and Ope (surgery under sevoflurane) groups. Then, the mice were divided into four groups: Naïve, Sevo (sevoflurane exposure), Ope, and Dex (dexmedetomidine injection before surgery). We selected the *Mapt* gene, the upregulated expression of which has been observed in our transcriptome analysis and in neurodegenerative disorders, as the target gene and investigated whether the changes in its expression occurred in the hippocampus using quantitative reverse transcription polymerase chain reaction (qRT-PCR). The cognitive function of mice was evaluated via the Barnes Maze test.

Results

In the qRT-PCR analysis, *Mapt* expression was significantly upregulated (2.60 ± 0.77 in mice from the Ope group vs. 1.00 ± 0.11 in mice from the Naïve group [mean \pm SD for fold change]; $p < 0.01$). Dexmedetomidine significantly attenuated the sevoflurane/surgery-induced upregulation of *Mapt* expression in the hippocampus (0.88 ± 0.32 ; $p < 0.01$). Sevoflurane/surgery-induced stress also increased the time to identify the target box, and dexmedetomidine treatment inhibited the time extension 7, 14, and 28 days after the surgery.

Conclusions

Dexmedetomidine attenuates the sevoflurane/surgery-induced cognitive deficit and *Mapt* expression in the hippocampus of aged mice.

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Key words: postoperative cognitive deficit, hippocampus, mRNA expression, transcriptome analysis, surgical stress, dexmedetomidine

1. Introduction

Postoperative cognitive dysfunction (POCD) is an important complication after surgery in clinical settings¹⁾. POCD has a negative impact on the quality of life of the affected patients and is more frequent and severe among elderly patients²⁻⁴⁾. In several human studies, general anesthetics or/and surgical procedures cause systemic inflammation⁵⁾, neuronal stress⁶⁾, cognitive dysfunction, and even increase mortality⁷⁾. In addition, aging may be the most critical risk factor for

POCD⁸⁻¹⁰⁾, but the details underlying the mechanism of POCD and its impact on brain networks are not fully understood.

Tau proteins, which are observed in neurodegenerative disorders, belong to the microtubule-associated protein tau (*Mapt*) family and play a significant role in the assembly of microtubules in during aging¹¹⁾. *In vitro*, plaque formation and tauopathy seen in Alzheimer's disease are influenced by anesthetic agents, suggesting that an interaction between general anesthesia and Alzheimer's disease pathogenesis may underlie

POCD¹²). However, whether *Mapt* regulation in the brain is directly associated with general anesthesia or/and surgical procedures in model mice with non-Alzheimer conditions has not yet been reported.

Using transcriptome analysis, we reported that the comprehensive messenger RNA (mRNA) profile of neurons in the mouse hippocampus changes drastically following the simple exposure to sevoflurane¹³. These changes in the hippocampus suggest that simple sevoflurane anesthesia may induce neuroinflammation. Le Freche et al. have demonstrated that simple sevoflurane exposure is also associated with spatial cognitive deficits and increased tau phosphorylation via the activation of specific kinases¹⁴. Some strategies and mechanisms for preventing cognitive deficits resulting from neuroinflammation and neurodegeneration have been reported. For example, high-mobility group box 1 (HMGB1) is upregulated in the brain and cerebrospinal fluid following surgery¹⁵, and the suppression of HMGB1 downregulates the expression of inflammatory pathway-related genes in aged rats¹⁶. Acetyl-L-carnitine may have neuroprotective effects on lipopolysaccharide (LPS)-induced neuroinflammation in mice via the regulation of brain-derived neurotrophic factor (BDNF)¹⁷.

Dexmedetomidine is a highly selective α_2 adrenergic agonist with effects on the human brain, including sedation, anesthesia, and analgesia^{18, 19}. Dexmedetomidine also has anti-inflammatory effects and suppresses inflammatory pathways²⁰. We hypothesized that the impairment of cognitive function due to surgery-induced inflammation and the mRNA expression change of several related genes in the hippocampus were alleviated by dexmedetomidine. This study aimed to verify the transcriptional network changes in the hippocampus of aged mice following sevoflurane/surgery-induced stress and to determine how dexmedetomidine affects postoperative cognitive function using the Barnes Maze test.

2. Material and Methods

2.1 Preparation of the animals

With approval from the Sapporo Medical University School of Medicine animal ethics committee (project number: 15-031), male C57/BL6 mice (10 weeks of age, body weight of about 25 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and housed at 22°C under controlled lighting conditions (12:12-hour light/dark cycle); they were provided free access to food and water. All mice used in this experimental protocol were housed under similar circumstances until they were

54-56 weeks of age and were then used for the experiments. All experimental treatments were performed following the animal guidelines of our institution, the National Institutes of Health Guide for the Care and Use of Laboratory animals²¹, and the International Association for the Study of Pain rules²².

2.2 Transcriptome analysis

Twelve aged male mice were assigned into two groups: (1) Naïve group (n = 6) and (2) Operation group (Ope group, n = 6) (Fig. 1A). The mice in the Naïve group were provided 100% oxygen alone for 1 hour after they were placed in a plastic chamber without being administered an anesthetic agent. The mice in the Ope group were anesthetized with 2.5% sevoflurane (Maruishi Co., Ltd. Shizuoka, Japan) for 1 hour and provided 100% oxygen; a 15-20-mm longitudinal incision was then made into their lower abdomen, followed by intestinal manipulation during sevoflurane anesthesia. The incision was sewn and closed using 6.0 VICRYL PLUS[®] sutures (Johnson and Johnson, NJ). No critical blood loss was observed during the surgery and incision-closure procedures. All anesthetized mice showed spontaneous breathing, and their oxygen saturation was observed to be maintained at over 93% when they were monitored with a MouseSTAT[®] Jr. system and a paw pulse oximeter sensor (Kent Scientific CORPORATION, CT). Their temperatures were monitored using a rectal probe and were observed to be maintained at over 37.0°C during the treatments. Then, the mice were decapitated under sevoflurane anesthesia, and the brain of each mouse was immediately removed from the skull, frozen at -70°C with 2-methylbutane, and placed in a Petri dish containing ice-cold phosphate-buffered saline. The brain was cut along the longitudinal fissure of the cerebrum, and the regions posterior to the lambda were cut off using tissue matrices (Brain Matrices, EM Japan, Tokyo, Japan). Thereafter, the brain was placed with the cortex of the left hemisphere facing down, and any non-cortical forebrain tissues were removed. Hippocampal blocks were obtained using Brain Matrices (EM Japan). Meningeal tissues were removed from the hemispheres according to a previously described method²³. Finally, dissected hippocampal cells were homogenized and lysed, to prepare six RNA samples from each mouse using the RNeasy[®] Plus Micro Kit (Qiagen, Hilden, Germany) and QIAcube (Qiagen). Quality control for the isolated RNA samples was performed using the Agilent 2200

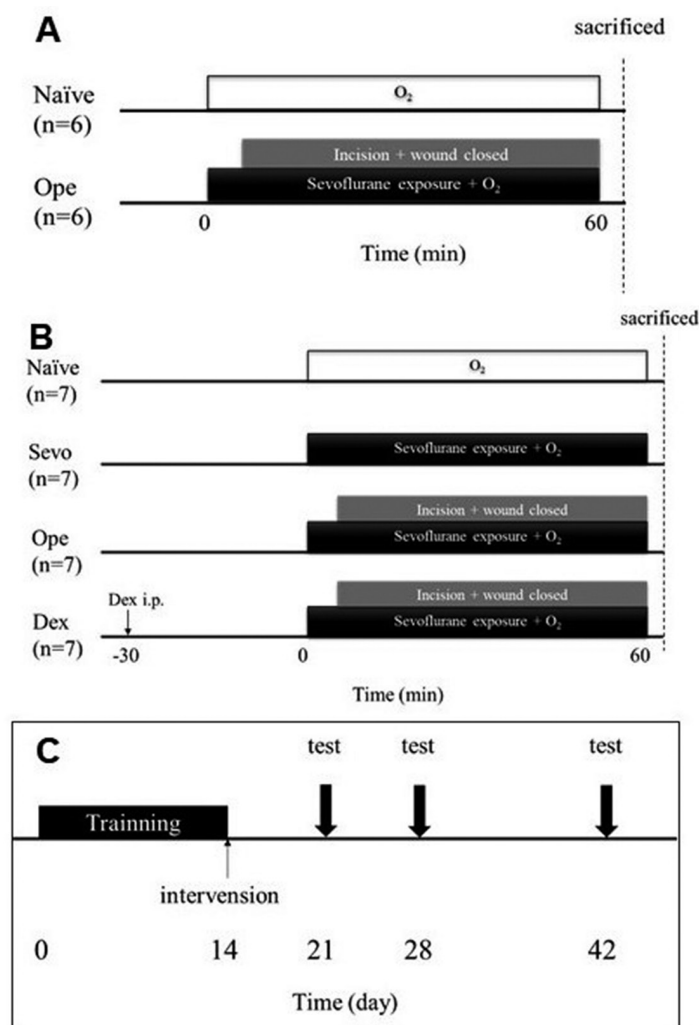


Figure 1. The protocol for the preparation of the animals. (A) Mice from both the Naïve and Ope groups were sacrificed after 60 minutes of the treatments, including the interventions, and the hippocampal sections of mice from each group were used for transcriptome analysis. (B) Similar to the protocol for transcriptome analysis, the mice from each group were sacrificed after 60 minutes of the treatments, and the hippocampal sections of the mice from the four groups were used for quantitative reverse transcription polymerase chain reaction. (C) Mice from each group were trained for 14 days and subjected to intervention. On the 7th, 14th, and 28th day after each intervention, a spatial test was performed using the Barnes Maze tracking system.

TapeStation system (Agilent Technologies, Santa Clara, CA). For the samples to pass the initial quality control step, the following criteria were used: > 1 µg of sample was obtained, and the equivalent RNA integrity number (eRIN) was ≥ 8 . The eRIN was determined using a 2500 Bioanalyzer Instrument, which provides accurate information²⁴. The isolated RNA was then pooled into two samples per group and labeled. A complementary DNA (cDNA) library was prepared using TruSeq[®] RNA Library Prep Kits (Illumina, Inc., San Diego, CA), according to the manufacturer's instructions. RNA-seq was performed in the paired-end (101 cycles x 2) mode on an Illumina HiSeq 2500 platform (Illumina, Inc.). Base call (.bcl) files for each cycle of sequencing were generated using the Illumina Real Time Analysis software (Illumina,

Inc.) and were analyzed primarily and de-multiplexed into a FASTQ file using Illumina's BCL2FASTQ conversion software (ver. 1.8.4, Illumina, Inc.). Raw paired-end RNA-seq reads in FASTQ formats were assessed for base call quality, cycle uniformity, and contamination using FastQC (<http://www.bioinformatics.bbsrc.ad.uk/projects/fastqc/>). Mapping of the quality control-filtered paired-end reads to the mouse genome and quantification of the expression level of each gene were performed using R software (ver. 3.1.1 with TCC package)^{25, 26}. The quality control-filtered paired-end reads were mapped to the public mouse genome data published by UCSC (NCBI37/mm9, <http://genomes.UCSC.edu/>).

2 • 3 Quantitative reverse transcription polymerase chain reaction (qRT-PCR)

A separate set of mice was divided into four different experimental groups: (1) Naïve group (n = 7), (2) sevoflurane-exposure group (Sevo group, n = 7), (3) Ope group (n = 7), and (4) dexmedetomidine (Maruishi Pharmaceutical, Osaka, Japan)-injection group (Dex group, n = 7) (Fig. 1B). The mice in the Naïve and Ope groups were treated as described for the transcriptome analysis procedure. The mice in the Sevo group were anesthetized with 2.5% sevoflurane for 1 hour in the presence of 100% oxygen after being placed in a plastic chamber. The mice in the Dex group were intraperitoneally (i.p.) administered with 10 µg/kg dexmedetomidine diluted in 0.1 ml of normal saline 30 minutes before the operation. The total RNAs from the mice in each group were isolated from the hippocampi of their frozen brain tissues by using an RNeasy Mini Kit (Qiagen), according to the manufacturer's standard protocol. The concentration and purity of the isolated RNA samples were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). cDNAs were generated by reverse transcription PCR using the QuantiTect[®] Reverse transcription Kit (Qiagen). For qRT-PCR, an SYBR Green QuantiTect Primer Assay (Qiagen, ID: QT00100170) specific for *Mapt* was used to quantify the mRNA expression levels, according to the manufacturer's instructions. Target gene expression was analyzed using the comparative CT method, using *Gapdh_3_SG* (Qiagen, ID: QT01658692) as the control²⁷⁾.

2 • 4 Behavioral test

Cognitive function before and after the intervention was evaluated via the Barnes Maze test by using a computerized video tracking system²⁸⁾. Training was performed for 14 days. For the training session, the animals were maintained in the same housing conditions throughout the test. The room temperature was maintained at 23°C, and the room was kept quiet during the test. In all trials, the mice were first placed in the middle of a circular platform with 20 equally spaced holes. One of these holes was connected to a dark chamber called the target box. Once the mouse entered the target box, the trial was finished, and the mouse was allowed to stay in the target box for 30 seconds. If the mouse failed to find the target box within 180 seconds, it was manually guided to the target box and allowed to remain in the hole for 30 seconds. Two trials were conducted per day for 14 days, and the target box remained at the same location throughout the test. The amount of time spent

finding and entering the target box (latency: seconds), the distance traveled to enter the box (distance: cm), and percent time in the quadrant were determined from the video tracking system (Lime Light 4, ACTIMETRICS, Wilmette, IL). The memory test was performed after the respective treatments at three-time points: postoperative days 7, 14, and 28. (Fig. 1C)

2 • 5 Statistical analysis

For transcriptome analysis, sets of genes showing differential expression were filtered to remove those with fold changes < 1.5 (upregulated or downregulated) and with a false discovery rate-corrected p value of > 0.05. The sample size was calculated with the following parameters: power ≥ 0.8, probability level < 0.05, and anticipated effect size = 14. In the qRT-PCR analysis, data were analyzed using one-way ANOVA with post-hoc Bonferroni correction. For the Barnes Maze test, the data were expressed as the mean ± standard deviation (S.D.). The behavioral data were analyzed using repeated ANOVA, followed by the Student-Newman-Keuls multiple-range test for post hoc comparisons. All analyses were performed using a statistical software (GraphPad Prism 6, GraphPad, San Diego, CA). p < 0.05 was considered statistically significant for all the experiments.

3. Results

3 • 1 Transcriptome Analysis

Four RNA samples from mice in each group had a quality of ≥ 1 µg and an eRIN value of ≥ 8. We investigated the changes in the expression levels of a total of 37,682 genes. A total of 2,786 genes were filtered because of little change in their mRNA expression levels. Microarray plotting revealed a total of 943 genes that showed differential expression among the mice from the two groups (Fig. 2). We detected 752 upregulated and 191 downregulated genes. Table 1 shows the top 20 genes that were highly upregulated after sevoflurane/surgery-induced stress. Among them, the isocitrate dehydrogenase 3 (NAD+) beta (*Idh3b*) gene was the most upregulated gene. This gene encodes the β subunit of isocitrate dehydrogenase 3, a mitochondrial enzyme of the tricarboxylic acid cycle. Besides, *Idh3b* encodes a possible apoptogenic factor that functions much like the cytochrome c released in mammalian cells and mediates cell death^{29, 30)}. *Mapt*, which is the focus of this study, showed a log2 ratio of 3.38, indicating that it was highly upregulated. Table 2 shows the top 20 most highly downregulated genes. The gene encoding late cornified envelope 3D (*Lce3d*) was the most downregulated gene.

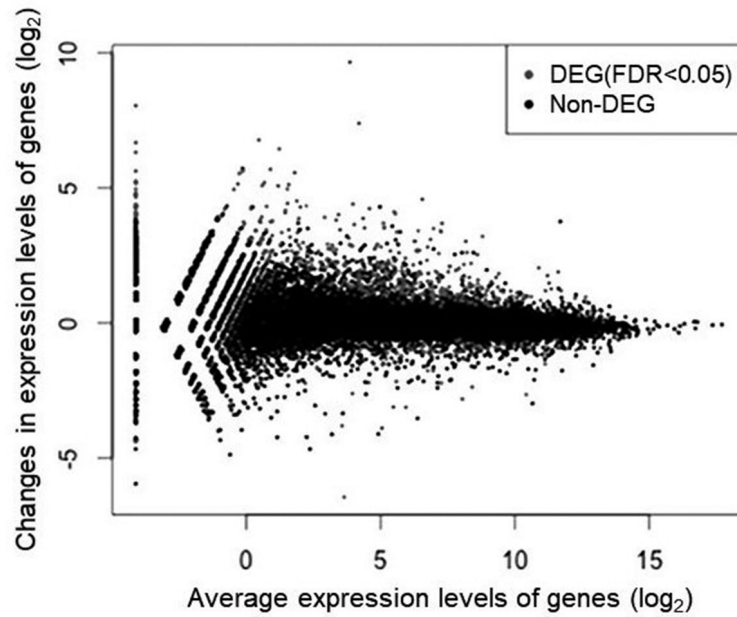


Figure 2. Changes in the expression levels of genes using microarray plotting. The horizontal axis shows the average expression levels of genes in samples from mice in the Naïve and Ope groups. The vertical axis shows the tendency of gene expression in samples from mice in the Ope group, compared to those from mice in the Naïve group. The gray circles represent the differentially expressed genes (DEGs) and the black circles represent the non-DEGs.

Table 1. Top 20 genes that were highly upregulated in the hippocampi of aged mice following surgery and sevoflurane exposure.

Gene name	Gene description	log2 ratio
Idh3b	isocitrate dehydrogenase 3(NAD+) beta	9.64
Tmpo	thymopoietin	8.05
Galnt11	UDP-N-acetyl-alpha-D-galactosamine: polypeptide N-acetylgalactosaminyltransferase 11	7.38
Prkab1	protein kinase, AMP-activated, beta 1 non-catalytic subunit	6.78
Cbx7	chromobox7	6.67
Hcn1	hyperpolarization-activated, cyclic nucleotide-gated K+1	6.44
Synpo2	synaptopodin2	6.31
Syt2	synaptotagmin2	5.72
Xlr	X-linked lymphocyte-regulated	5.68
Kend3	potassium voltage-gated channel, Shal-related family, member 3	5.62
Il1f5	interleukin 1 family, member 5 (delta)	5.61
Arfg2	ArfGAP with FG repeats 2	5.56
Ttc19	tetratricopeptide repeat domain 19	5.25
Cox7b2	cytochrome c oxidase subunit 7b2	5.18
Wdr37	WD repeat domain 37	5.18
Gm5431	predicted gene 5431	5.15
Ippk	inositol 1,3,4,5,6-pentakisphosphate 2-kinase	5.12
Gpr135	G protein-coupled receptor 135	5.09
Psm1	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	5.08
Bst2	bone marrow stromal cell antigen 2	4.96

Table 2. Top 20 genes that were highly downregulated in the hippocampi of aged mice following surgery and sevoflurane exposure.

Gene name	Gene description	log2 ratio
Lce3d	late cornified envelope 3D	-6.44
Rtn4r	reticulon 4 receptor	-5.95
Mansc1	MANSC domain containing 1	-4.86
Zbed3	zinc finger, BED type containing 3	-4.67
2010106E10 Rik	RIKEN cDNA 2010106E10 gene	-4.66
Trmt2b	TRM2 tRNA methyltransferase 2B	-4.36
Cldn5	claudin 5	-4.33
Chia1	chitinase, acidic 1	-4.32
Actl7b	actin-like 7b	-4.28
Arl4d	ADP-ribosylation factor-like 4D	-4.24
Mir299b	micro RNA 299b	-4.21
Uqcrc2	ubiquinol cytochrome c reductase core protein 2	-4.11
Gpr182	G protein-coupled receptor 182	-3.99
Hoxb5	homeobox B5	-3.96
Spta11	spectrin alpha, erythrocytic 1	-3.96
Cdv3	carnitine deficiency-associated gene expression in ventricle 3	-3.89
Tmed7	transmembrane p24 trafficking protein 7	-3.79
AF366264	cDNA sequence AF 366264	-3.7
Tsga10	testis specific 10	-3.68
Fcgr4	Fc receptor, IgG, low affinity IV	-3.53

3·2 qRT-PCR

Mapt is a neuronal protein that is highly enriched in axons and is important for neuronal development and the control of neurological degeneration. *Mapt* was highly upregulated in mice from the Ope group, compared to the case for those from both the Naïve and Sevo groups ($p < 0.01$). *Mapt* was remarkably downregulated in the mice from the Dex group, compared to the case for those from the Ope group ($p < 0.01$) (Fig. 3).

3·3 Barnes Maze test

We found no difference between the cognitive function of the mice from the different groups on day 1. The time taken (seconds) and the distance travelled (cm) to enter the target box during the 14-day training sessions in the Barnes maze test gradually decreased in case of mice from all the groups (Fig. 4 and Fig. 5). The time taken and distance traveled to enter the target box on day 14 were significantly lower than those on day 1. When the cognitive function of the mice was tested on postoperative day seven after the training sessions, the time spent by the mice subjected to surgery to identify the target box was longer than that in case of the mice from the other three groups. However, this increase in the time was attenuated by dexmedetomidine intervention. A similar pattern was observed on postoperative day 14 after the training sessions. On

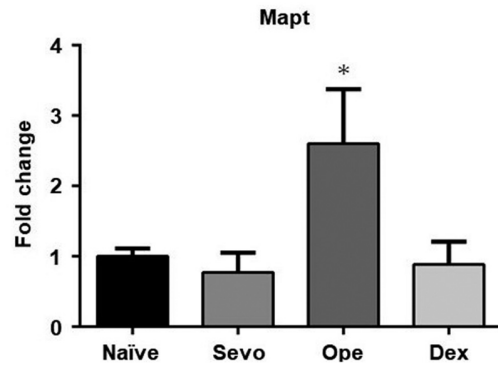


Figure 3. The fold change of *Mapt* expression in samples from mice in the Naïve, Sevo, Ope, and Dex groups. The expression level of *Mapt* is shown as the mean \pm SD. In the samples from mice in the Ope group, the fold change was 2.60 ± 0.77 . The post hoc-adjusted p value is $*p < 0.01$ versus other groups. The mice in the Sevo group were only exposed to sevoflurane, those in the Ope group underwent surgery during sevoflurane exposure, and those in the Dex group received dexmedetomidine intraperitoneally before the surgery.

postoperative day 28, the time taken by the mice from the Ope group to enter the box was longer than that taken by the mice from the other groups to enter the box. On days 7 and 14 after the training sessions, the distance traveled by the mice from the Ope group to enter the target box was significantly longer than that in case of the mice from the other three groups. The

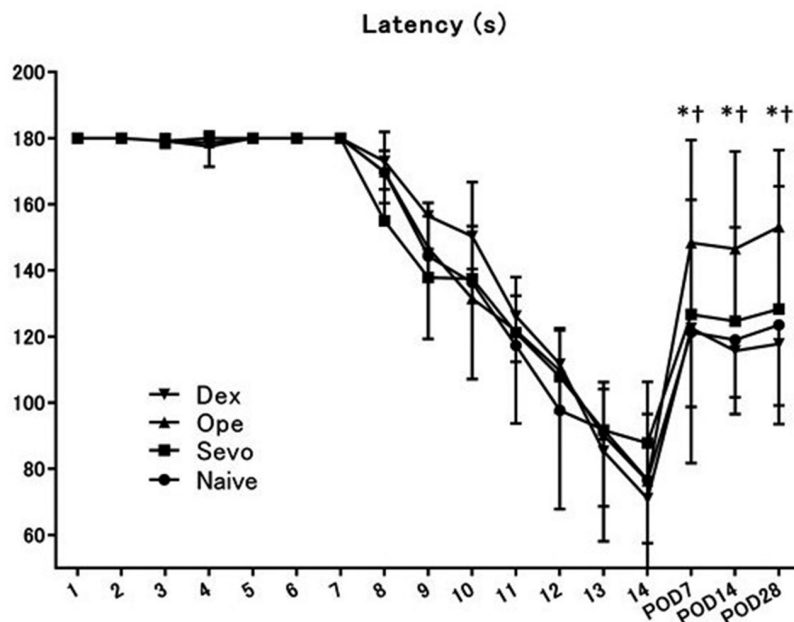


Figure 4. Changes in latency (seconds) from the training to the test session. After 14 days of the training sessions, the time spent to identify the target box (seconds) gradually decreased. On postoperative days 7, 14, and 28, the mice in the Ope group needed more time to find the hole compared to those from the other groups ($*p < 0.05$). Dexmedetomidine alleviated the sevoflurane/surgery-induced cognitive deficit. The cognitive function of mice from each group is exacerbated compared to that on the 14th training day ($\dagger p < 0.05$).

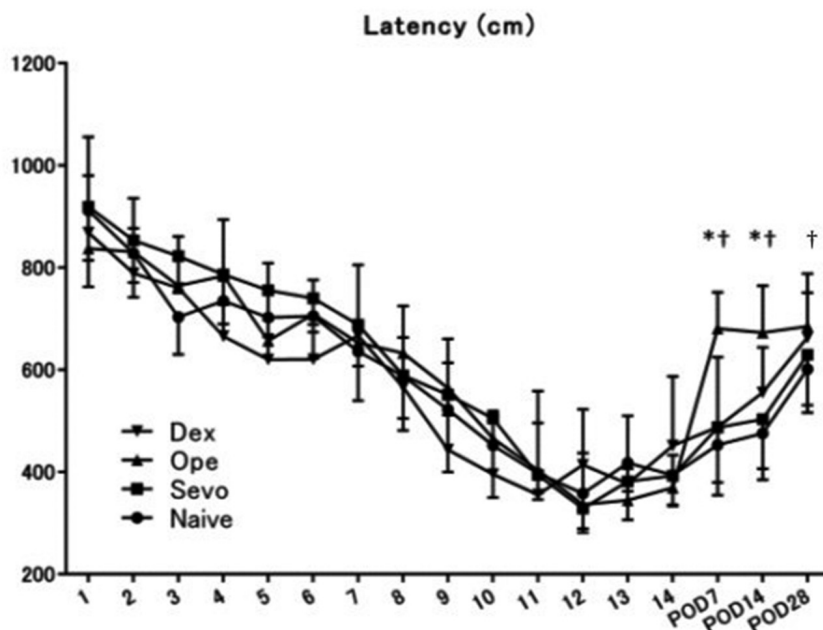


Figure 5. Changes in latency (distance) from the training to the test session. After 14 days of the training sessions, the distance travelled to identify the target box (cm) gradually decreased. On postoperative days 7 and 14, the mice in the Ope group travelled a longer distance to find the hole compared to those in the other groups ($*p < 0.05$). Dexmedetomidine alleviated the sevoflurane/surgery-induced cognitive deficit. The cognitive function of mice from each group is exacerbated compared to that on the 14th training day ($†p < 0.05$).

percent time spent in the quadrant with the target box was not significantly different among the mice from the different groups (Data not shown.).

4. Discussion

4.1 The aged mouse model

The molecules in mice that regulate cell differentiation and death are similar to those in humans³¹. Moreover, the aging process between mice and humans is also similar³². The findings obtained from the experiments using aged mice can be considered similar to the changes that occur in elderly humans. In this experimental protocol, we housed all mice under similar circumstances until they were 54-56 weeks of age, which is equivalent to the conditions observed in at least middle-aged or older humans.

4.2 Transcriptome analysis

Recent progress in genomics has enabled us to comprehensively analyze cellular modifications at the gene expression level. The DNA microarray technique has revealed various mechanisms underlying diseases; however, only a few investigations of the association between POCD and the hippocampus have been performed via a transcriptome-wide association study. Transcriptome analysis can capture both coding and non-coding RNAs

and quantify the heterogeneity of gene expression using samples from various tissues. This analysis has some advantages³³. Functional characterization and annotation of genes are possible, and researchers can reconstruct genetic interaction networks³⁴ and identify clues related to disease processes and prognoses^{35, 36}. In our experimental protocol, as the first step, we performed whole-transcriptome analysis to investigate the differences between the gene expression in the hippocampi of mice from the Naive and Ope groups. In a clinical study involving humans, Peffer et al. have demonstrated that drastic changes in RNA occur at numerous genomic levels, indicating that the regulation of transcriptional networks is involved in aging-associated degeneration³⁷. Additionally, Broad et al. have revealed that surgery-induced cell death is significantly increased in the brains of developing piglets. Using RNA sequence data, this group has identified changes in the expression of gene transcripts following the administration of isoflurane anesthesia alone, compared to that following surgery³⁸. These findings indicate that transcriptome analysis is useful for investigating differences in gene expression before and after an intervention, and can identify the association between these changes and the mechanisms underlying these phenomena. In our study, we detected all the upregulated and downregulated

genes whose expression levels were affected by surgery-induced stress, and identified several target genes that may be related to postoperative cognitive deficits. According to the transcriptome analysis in this study, *Mapt* may be an important target gene that is strongly associated with sevoflurane/surgery-induced stress.

4.3 *Mapt*

Mapt is expressed in the central nervous system and encodes the tau protein. The tau protein is necessary for maintaining axonal function, and it plays a role in axonal transport^{39,40}, synaptic plasticity^{41,42}, and nucleic acid protection^{43,44}. However, once abnormal external stress and neuronal cell death occur in the brain, the tau protein accumulates, causing tauopathies, which are one of the causes of, and pathogenic mechanisms underlying, neurodegenerative diseases such as Alzheimer's disease,⁴⁵ frontotemporal lobar degeneration (FTLD)^{46,47}, and post-traumatic brain injury⁴⁸. The influence of tauopathies on cognitive function appears to be strong in aged individuals and is the reason for the decrease in cerebral metabolism in elderly people. From the results of this study, we suggest two possibilities for the significant increase in the *Mapt* expression level in the hippocampi of the aged mice. The *Mapt* expression may have increased directly as a consequence of the surgical procedure and/or indirectly due to the suppression of the metabolism in the brain. The expression level of *Mapt* may be associated with the pathogenesis of behavioral dysfunction, and serves as a biomarker in the hippocampus of mice, indicating sevoflurane/surgery-induced cognitive deficit. In a surgical model, under general anesthesia, systemic inflammation spreads in the brain and affects the expression level of *Mapt*. We suppose that short-term surgical stress may lead to changes in the hippocampus that are similar to those observed in case of age-related degeneration.

4.4 Dexmedetomidine inhibits sevoflurane/surgery-induced upregulation of *Mapt* expression

Dexmedetomidine, a selective α_2 adrenergic receptor agonist, is used clinically for perioperative sedation and anesthesia^{49,50}. In addition, dexmedetomidine elicits anti-inflammatory effects via the modulation of several pathways, such as the cholinergic anti-inflammatory pathway and the NF- κ B pathway^{51,52}, and suppresses postoperative agitation and emergence delirium. Paeschke et al. have investigated whether the gene expression of interleukin-1 β (IL-1 β) and tumor necrosis factor- α

(TNF- α), and the expression of the microRNAs miR124, 132, 134, and 155 in the adult rat hippocampus, cortex, and plasma changed after the intraperitoneal administration of 1 mg/kg LPS in the presence or absence of 5 μ g/kg dexmedetomidine⁵³. Dexmedetomidine significantly suppressed LPS-induced neuroinflammation in the hippocampus and cortex via the reduction of the IL-1 β and TNF- α gene expression after 24 hours, and drastically decreased the expression of microRNAs in both these regions of the brain. Moreover, dexmedetomidine elicits a direct neuroprotective effect via BDNF induction and an indirect neuroprotective effect by promoting the release of BDNF from astrocytes⁵⁴.

In our study, we injected 10 μ g/kg dexmedetomidine into the mice from the Dex group before the surgery. All mice received a mild sedative without down saturation. In previous studies, the intensity of invasive stress and the severity of inflammation have been reported to be different, and the dexmedetomidine doses administered also varied. Xiong et al. have stated that 12 μ g/kg represents a high dose, and 3 μ g/kg represents a low dose of dexmedetomidine⁵⁵. Thus, the dose of dexmedetomidine that we used in our protocol (10 μ g/kg) was quite reasonable. Dexmedetomidine may attenuate the upregulation of *Mapt* expression via several pathways and elicit a neuroprotective effect.

4.5 Dexmedetomidine may attenuate sevoflurane/surgery-induced cognitive deficit

This study demonstrated that dexmedetomidine attenuated sevoflurane/surgery-induced cognitive deficit by downregulating the *Mapt* expression. This result may explain one of the possible mechanisms underlying the effects of dexmedetomidine and become a key to prevent cognitive deficit. The results of the Barnes Maze test showed that the latency in case of the mice from the Ope group was significantly longer than that in case of the mice from the Naïve group on the 7th, 14th, and 28th day after surgery, and that the distance travelled by the mice from the Ope group was significantly longer than that travelled by the mice from the Naïve group on the 7th and 14th day after surgery. Dexmedetomidine intervention prior to the open abdominal surgery shortened the time latency in case of the mice from the Ope group on the 7th, 14th, and 28th day after surgery, and the distance travelled on the 7th and 14th day after surgery. These results suggested that dexmedetomidine may be effective for improving the sevoflurane/surgery-induced cognitive

deficit or for maintaining cognitive function following surgery-induced stress. Xiong et al. have also reported that dexmedetomidine may limit the degree of expression of relaxin-3 and the overexpression of c-fos in the hippocampus, downregulate the expression of pro-apoptosis proteins such as Fas, caspase-8, and caspase-9, and upregulate the expression of the anti-apoptosis protein Bcl-2⁵⁵). Yamanaka et al. have also shown that early dexmedetomidine treatment attenuates these systemically induced neurocognitive changes by preventing subsequent hippocampal neuroinflammation and the overexpression of TLR-4 in the microglia⁵⁶). Our results are consistent with these previous results.

4 • 6 Limitations

This study has some limitations. We observed only the mRNA expression changes in the hippocampus and did not investigate the related downstream pathways. We focused on the fluctuation of *Mapt* expression in cognitive dysfunction; however, other genes may be involved in this process as well. Indeed, the results of the transcriptome analysis showed that many genes were upregulated; however, we could not clearly identify which gene is the most important. *Mapt*, which has been suggested to be relevant to cognitive function in this study, was the only clue uncovered in this study. To confirm the sequential phenomena caused by dexmedetomidine injection, we need to perform the same protocol in the *Mapt*-knockout mice.

In this experimental protocol, we observed relatively early behavioral changes at 7, 14, and 28 days after the intervention. Based on the results of this study, we could not conclude whether these behavioral changes were caused by postoperative delirium or cognitive dysfunction, because we could not precisely define the boundary between these phenomena.

The analgesic effect of dexmedetomidine may have influenced the behavioral changes after intervention. However, since both the blood and tissue concentrations of dexmedetomidine were not examined in this experimental protocol, it is impossible to demonstrate how dexmedetomidine directly affects cognitive behavior after the intervention. In this experimental protocol, transcriptome analysis of the samples from the mice in the Dex group was not performed, and only the comparison between the samples from mice in the Naïve and Ope groups is conducted; a standard transcriptome analysis method to examine the samples

from mice in multiple groups has not been established. In order to clarify the mechanisms underlying the effects of dexmedetomidine, the transcriptome analysis of the samples from mice in the Dex group may also be needed.

5. Conclusions

In conclusion, dexmedetomidine attenuates sevoflurane/surgery-induced cognitive deficit and the hippocampal *Mapt* expression in aged mice. Although further experiments and analyses are needed to confirm these results, our findings suggest that POCD may be attenuated by suppressing the *Mapt* expression in the hippocampus.

6. Acknowledgments

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7. Abbreviations

POCD: postoperative cognitive dysfunction, *Mapt*: microtubule-associated protein tau, HMGB1: high-mobility group box 1, LPS: lipopolysaccharide, BDNF: brain-derived neurotrophic factor, eRIN: equivalent RNA integrity number, i.p.: intraperitoneally, IL-1 β : interleukin-1 β , TNF- α : tumor necrosis factor- α

Author contributions

Shunsuke Tachibana developed a research plan, conducted the experiments, and wrote this manuscript. Tomo Hayase helped design the experiment, and also analyzed the data and revised the manuscript. Michiaki Yamakage made critical revisions to the manuscript and contributed towards the writing the manuscript. All authors have approved the final manuscript.

Disclosure

All authors disclose no financial and personal relationships with other people or organizations that could potentially and inappropriately influence this research and conclusions. A Grant-in-Aid for Young Scientists (B) supported this work (No.15K20051, 2015-2018, to Shunsuke Tachibana).

Conflict of interest

The authors declare no competing financial interests.

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デクスメドミジンは、高齢マウスにおいて、セボフルラン暴露下における手術侵襲由来の認知機能低下を減弱し、*Mapt* 遺伝子の発現を抑制する

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目的

麻酔・手術後の高齢者における術後認知機能低下は、重要な合併症のひとつであるが、その詳細な機序や脳内ネットワークにおける影響に関して、完全に明らかにはなっていない。本研究においては、高齢手術マウスモデルを作製・確立した上で、鎮静作用や抗炎症作用を有する $\alpha 2$ アドレナリン受容体アゴニスト、デクスメドミジンの投与が、マウス海馬においてどのような変化をもたらし、実際の認知機能にどのように反映するかを調べた。

方法

はじめに、麻酔・手術侵襲が高齢マウス海馬にどのような mRNA 変化をもたらさるかトランスクリプトーム解析を行なった。Naive 群とセボフルラン暴露下での手術群を検討し、手術侵襲によって発現が大きく変化したものを候補遺伝子とした。次に複数の候補遺伝子の中から、神経変性疾患にも関与するとされる *Mapt* に注目して、介入によってどのような変化があるかを調べた。Naive 群、セボフルラン暴露群、手術群（セボフルラン暴露下）、デクスメドミジン群（セボフルラン暴露下での手術前にデクスメドミジン

を投与）の 4 群に分類し、qRT-PCR で海馬における *Mapt* 発現の変化を比較検討した。また、同 4 群に関してバーンズメイズ試験を用いて、認知機能の変化を評価した。

結果

トランスクリプトーム解析では、麻酔・手術侵襲によって 752 種類の mRNA 由来の遺伝子が発現を増大させ、191 種類の遺伝子が発現を減少させたことが明らかとなった。qRT-PCR では、海馬における *Mapt* の発現量は手術群において有意に増大した（手術群 2.60 ± 0.77 vs Naive 群 1.00 ± 0.11 [mean fold change \pm SD], $p < 0.01$)。バーンズメイズ試験では、麻酔・手術侵襲によってターゲットボックス認知までの時間は延長したが、デクスメドミジン投与によって認知時間延長が抑制された。

結論

デクスメドミジンは、セボフルラン暴露下での手術侵襲による認知機能低下を減弱し、かつ、高齢マウスの海馬における *Mapt* 発現を抑制する。