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Dexmedetomidine attenuates sevoflurane/surgery-induced cognitive deficits and inhibits expression of *Mapt* in aged mice

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ABSTRACT

Background: Postoperative cognitive dysfunction is an important complication of surgery but the mechanism and impact on brain network are not fully understood. Dexmedetomidine, a highly selective α2 adrenergic agonist, has anti-inflammatory effects on the brain. The goal of this study was to elucidate how transcriptional network changes in the hippocampus of aged mice exposed to sevoflurane/surgery-induced stress and to determine how dexmedetomidine impacts cognitive function.

Methods: Mice were divided into four groups- Naïve, Sevo (sevoflurane exposure), Ope (surgery under sevoflurane) and Dex (dexmedetomidine injection before surgery). We focused on *Mapt* as the target gene, because *Mapt* is important in neurodegenerative disorders. We investigated whether *Mapt* expression changes occurred in the hippocampus of each group using quantitative real-time polymerase chain reaction (qRT-PCR). Cognitive function before and after the intervention was evaluated with the Barnes Maze test.

Results: qRT-PCR analysis showed that the level of *Mapt* expression was significantly up-regulated (2.60 ± 0.77 in the Ope group, 1.00 ± 0.11 in the Naïve group and 0.77 ± 0.28 in the Sevo group [mean fold change ± SD], respectively. $p < 0.01$). Dexmedetomidine treatment inhibited the *Mapt* expression induced by sevoflurane/surgery (0.88 ± 0.32, $p < 0.01$). In the Barnes Maze test, sevoflurane/surgery stress also increased the time to identify the target box and dexmedetomidine treatment inhibited the time extension when tested 7, 14, and 28 days after the training sessions.

Conclusion: Our results suggest that dexmedetomidine attenuates sevoflurane/surgery-induced cognitive deficits and inhibits the *Mapt* expression in the hippocampus of aged mice.

POSTOPERATIVE cognitive dysfunction (POCD) is an important complication after a surgery in clinical settings.[1] POCD has a negative impact on the quality of life in affected patients, and is seen in elderly patients more frequently and severely.[2-4] In several human observation studies, general anesthetics or/and surgical procedures cause systemic inflammation, neuronal stress, cognitive dysfunction and even increase the mortality.[5-7] In addition, aging may be the most important risk factor for POCD, but the details of the mechanism of POCD and the impact on brain networks are not fully understood.[8-10]

Tau proteins observed in neurodegenerative disorders belong to the family of microtubule-associated protein tau (Mapt) and play a significant role in assembly of microtubules during aging.[11] In vitro, plaque formation and tauopathy seen in Alzheimer disease are influenced by anesthetic agents, suggesting that an interaction between general anesthesia and Alzheimer pathogenesis may underlie POCD.[12] However, the study of *Mapt* regulation in the brain in direct association with general anesthesia or/and surgical procedures in non-Alzheimer model mice has not been reported.

Using transcriptome analysis, we reported that the comprehensive messenger RNA (mRNA) profile of neurons in the mouse hippocampus is dramatically changed following simple exposure to sevoflurane.[13] These changes in brain suggest that simple sevoflurane anesthesia may induce neuroinflammation. Le Freche et al. demonstrated that simple sevoflurane exposure is also associated with spatial cognitive deficits and increased tau phosphorylation via activation of specific kinases.[14]

Strategies are needed to prevent cognitive deficits derived from neuroinflammation and neurodegeneration. For example, high mobility group box 1 (HMGB1) is up-regulated in the brain and cerebrospinal fluid after surgery, suppressing HMGB1 down-regulates expression of inflammatory pathway genes in aged rats.[15,16] Acetyl-l-carnitine may have neuroprotective effects on lipopolysaccharide (LPS)-induced neuroinflammation in mice via regulation of brain-derived neurotrophic factor (BDNF).[17]

Dexmedetomidine is a highly selective α2 adrenergic agonist with effects on the human brain, including sedation, anesthetic effects, and analgesia.[18,19] Dexmedetomidine also has anti-inflammatory effects and suppresses inflammatory pathways.[20] We hypothesized that cognitive function changed along with *Mapt* expression due to surgery-induced inflammation and that dexmedetomidine improves cognitive behavior. Furthermore, we assumed that these changes are more pronounced with age. The aims of this study were to verify transcriptional network changes in the hippocampus of aged mice with sevoflurane/surgery-induced stress and to determine with the Barnes Maze test how dexmedetomidine affects cognitive function.

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Materials and Methods

Animal treatment

With approval from the Sapporo Medical University School of Medicine animal ethics committee (project number: 15-031) for this study, male C57BL/6 mice (10 weeks of age, body weight about 25 g) were purchased from Japan SLC, Inc. (Hamamatsu, Japan) and housed at 22˚ C under controlled lighting (12:12-hour light/dark cycle) with food and water available without limitation. All mice used in this experimental protocol were housed under similar circumstances until 54-56 weeks of age and were then used in the experiment. All experimental treatments were performed in accordance with the National institutes of Health Guide for the Care and Use of Laboratory animals, and the international association for the study of Pain rules.[21,22]

Transcriptome analysis

Twelve aged male mice were assigned to two groups: Naïve group (n = 6) and operation group (Ope group, n = 6) (Figure 1A). In the Naïve group, only 100% oxygen was provided to mice in a plastic chamber for 1 hr without anesthetic agent. In the Ope group, the mice were anesthetized with 2.5% sevoflurane (Maruishi Co., Ltd. Shizuoka, Japan) for 1 hr in 100% oxygen, and a 15- to 20- mm longitudinal incision was made in the lower abdomen, followed by intestinal manipulation during sevoflurane anesthesia. The incision was sewn and closed with 6.0 VICRYL PLUS® (Johnson and Jonson, New Brunswick, NJ). Neither critical blood loss nor intestinal damage was observed during surgery and closure. All anesthetized mice breathed spontaneously and maintained oxygen saturation (SpO_2) over 93% during monitoring with MouseSTAT® Jr. and a paw pulse oximeter sensor (Kent Scientific CORPORATION, Torrington, CT). The temperature was monitored with a rectal probe and was maintained 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 Petridish containing ice-cold phosphate-buffered saline. The brain was cut along the longitudinal fissure of the cerebrum, and the regions posterior to 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 tissue was removed. Tissue blocks containing hippocampal cells were obtained using Brain Matrices (EM Japan). Meningeal tissue was removed from the hemisphere according to a previously described method.[23] Finally, dissected hippocampal cells were homogenized, lysed, and divided into six samples for each mouse using reagents from the RNeasy® Plus Micro Kit (Qiagen, Hilden, Germany) and QIAcube (Qiagen). Quality control for isolated RNA was performed using the Agilent 2200 TapeStation system (Agilent Technologies, Santa Clara, CA). For samples to pass the initial quality control step, > 1 μg of sample was obtained, and the equivalent RNA integrity number (eRIN) was ≥ 8. The eRIN was determined with a 2500 Bioanalyzer Instrument (Agilent Technologies,), which provide accurate information.[24] 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 with Illumina Real Time Analysis software (Illumina, Inc.) and were analyzed primarily and de-multiplexed into a FASTQ (.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. 3.4.3 with TCC package).[25,26] The quality control-filtered paired-end reads were mapped to public mouse genome data published by UCSC (NCBI37/mm9, http://genomes.UCSC.edu/).

Quantitative real-time polymerase chain reaction (qRT-PCR)

A separate set of mice was randomly divided into four different experimental groups: Naïve group (n = 7), sevoflurane exposure only group (Sevo group, n = 7), Ope group (n = 7) and dexmedetomidine (Maruishi Pharmaceutical, Osaka, Japan) injection group (Dex group, n = 7) (Figure 1B). Mice in the Naïve and Ope groups were treated described for as transcriptome analysis. In the Sevo group, 2.5% sevoflurane in 100% oxygen was administered to mice in a plastic chamber for 1 hr. In the Dex group, the mice were intraperitoneally (i.p.) administered 10 μg/kg dexmedetomidine diluted in 0.1ml normal saline 30 min before the operation. Total RNAs of each group were isolated from frozen brain tissues including the hippocampus using an RNeasy Mini Kit (Qiagen) according to the manufacturer's standard protocol. The concentration and purity of the isolated RNA were assessed using a NanoDrop 1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA). cDNAs were generated by reverse transcription PCR using QuantiTect® Reverse transcription Kit (Qiagen). For using qRT-PCR, the 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 (ΔΔCT) method with Gapdh_3_SG (Qiagen, ID: QT01658692) as the control.[27]

Behavioral test

Cognitive function before and after the intervention was evaluated with the Barnes Maze test by using a computerized video tracking system.[28] Training was performed for 14 days. For the training session, 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, 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 to enter the box (distance: cm), and percent time in the quadrant with the target box were determined from the recorded videos using video tracking system for Barnes Maze (Lime Light 4, ACTIMETRICS, Wilmette, IL). The memory test was performed after treatment- at three time points: post-operative days on 7, 14 and 28 (Figure 1C).

Statistical analysis

For transcriptome analysis, differential gene sets were filtered to remove those with fold changes < 1.5 (up or down-regulated) 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. For 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.). Behavioral data were analyzed using a repeated ANOVA, followed by a Student-Newman-Keuls multiple range test for post hoc comparisons. All analyses were performed using statistical software (GraphPad Prism 6, GraphPad, San Diego, CA). P value less than 0.05 were considered statistically significant in all procedures.

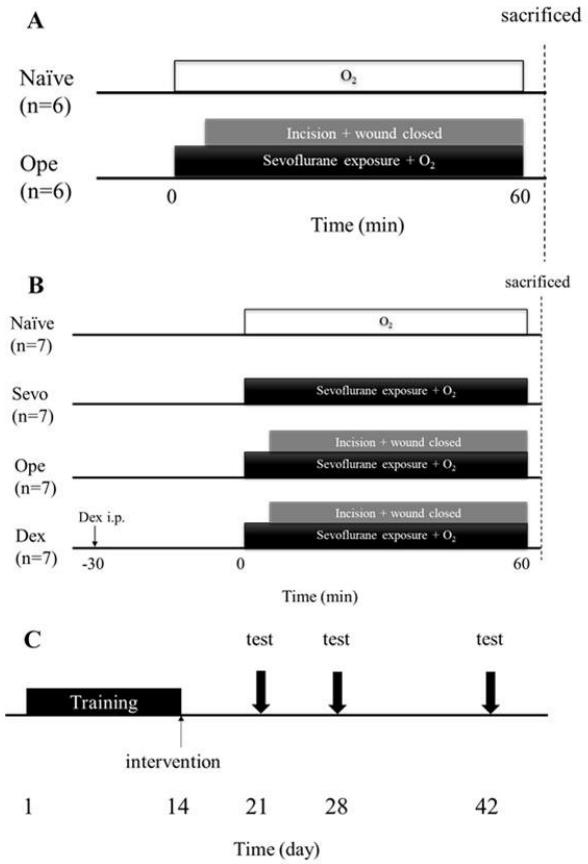

Fig. 1. The protocol for animal preparations. Mice in both the Naïve and Ope groups were sacrificed 60 min after treatments, and hippocampal sections of each group were used for transcriptome analysis (A). Similar to the protocol for transcriptome analysis, mice in each group were sacrificed 60 min after treatments and hippocampal sections of each group were used for quantitative real-time polymerase chain reaction (B). Mice in each group were trained for 14 days prior to the intervention. On the 7th , 14th , and 28th day after each intervention, a spatial test was conducted by the using Barnes Maze tracking system (C).

Results

Transcriptome analysis

Four RNA samples from each group had a quality ≥ 1 μg and eRIN value ≥ 8. We investigated changes in expression levels of total of 37,682 genes. A total of 2,786 genes were filtered because of little change in mRNA expression levels. Microarray plotting showed a total of 943 genes that were expressed differentially between the two groups (Figure 2). We detected 752 up-regulated genes and 191 down-regulated genes. Table 1 shows the top 20 genes that were highly up-regulated after sevoflurane/surgery stress. Among them, isocitrate dehydrogenase 3 (NAD+) beta (*Idh3b*) was the most highly up-regulated. This gene encodes the β subunit of isocitrate dehydrogenase 3, a mitochondrial enzyme of the tricarboxylic acid cycle.[29] In addition, *Idh3b* is suggested an apoptogenic factor that functions much like released cytochrome *c* in mammalian cells and

interact with cell death.[29,30] Thymopoietin (*Tmpo*) was the second most highly up-regulated in this transcriptome analysis. *Tmpo* causes an increase in ACTH production and release that are generated by following stress.[31] Synaptotagmin 2 (*Syt2*) was also ranked in the top 20 genes that were highly up-regulated. Synaptotagmins are transmembrane proteins involved in the regulation of membrane trafficking, showing changes of the expression in mouse model of Alzheimer disease that may be related to neuroinflammation surrounding the β -amyloid plaques.[32] *Mapt* which we focused on in this study, showed a $\log_2$ ratio of 3.38 (*i.e.*, highly up-regulated). Table 2 shows the top 20 most highly down-regulated genes. The gene encoding late cornified envelope 3D (*Lce3d*) was the most down-regulated gene.

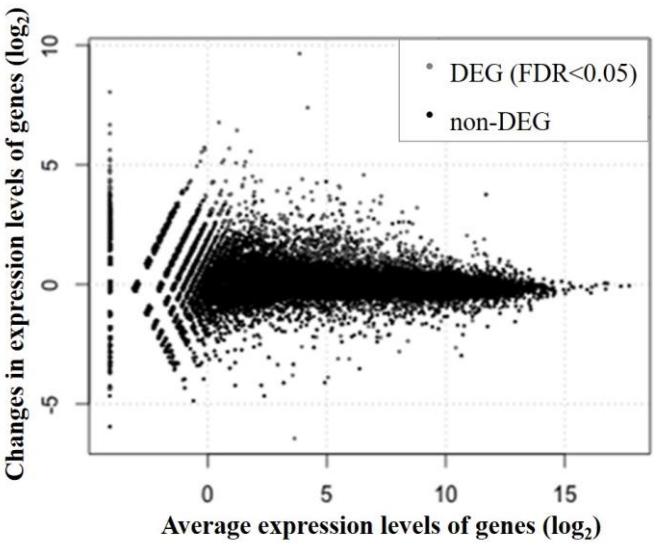

Fig. 2. Changes in the expression levels of genes using microarray plotting. The horizontal axis shows average expression levels in the Naïve and surgery (Ope) groups. The vertical axis shows the tendency of gene expression in the Ope group compared to the Naïve group. Gray circles represent differentially expressed genes (DEG), and black circles represent non-DEG.

Quantitative real-time polymerase chain reaction (qRT-PCR)

Mapt encodes a neuronal protein that is highly enriched in axons, and is important in neuronal development and control of neurological degeneration and this gene was highly up-regulated in the Ope group compared with both the Naïve and Sevo groups ($P < 0.01$). *Mapt* was not significantly different between the Naïve and Sevo groups. *Mapt* was remarkably down-regulated in the Dex group compared with the Ope group ($P < 0.01$) (Figure 3).

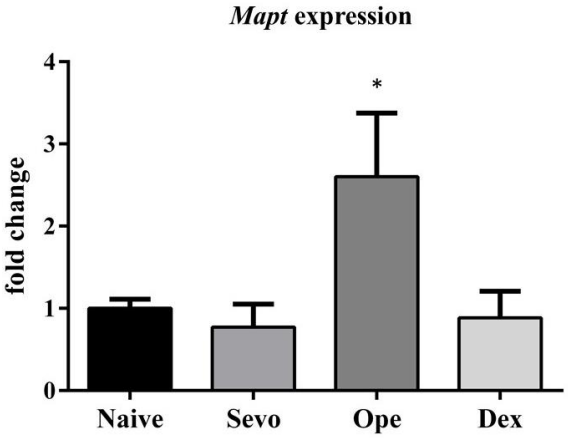

Fig. 3. The fold change in Mapt expression in Naïve, Sevo, Ope, and Dex groups. The expression level of Mapt is shown as the mean ± S.D. In the Dex group, the fold change was 2.60 ± 0.77. The post hoc-adjusted p value is *p < 0.01 versus other groups. The Sevo group was only exposed to sevoflurane, the Ope group underwent surgery during sevoflurane exposure, and the Dex group received dexmedetomidine i.p. before surgery.

Barnes Maze test

We found no difference in cognitive function among the groups on day 1. The time (seconds) and distance (cm) to enter the target box during the 14-day training sessions of the Barnes Maze test gradually decreased in all mouse groups (Figure 4 and 5). The time and the distance on day 14 were significantly lower than those on day 1. When the cognitive function of mice was tested on post-operative day 7 after the training sessions, the time to identify the target box for the mice subjected to surgery was longer than those of the other three groups. However, these increased times were attenuated by dexmedetomidine administration. On post-operative day 14, the prolongation also showed attenuation due to dexmedetomidine administration. On post-operative day 28, the time in the Ope group was longer than that in any other groups. On days 7 and 14 after training sessions, the distance in the Ope group was significantly longer than in the other three groups. The percent time in the quadrant with the target box was not significantly different among the groups.

Discussion

The aged mouse model

The molecules in mice that regulate cell differentiation and death are similar to those in humans.[33] Moreover, the aging process is also similar in mice and humans.[34] 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 in similar circumstances until 54-56 weeks of age, which is equivalent to at least middle-aged or older humans.

Table1. Top 20 genes that were are highly up-regulated in aged mouse hippocampus by 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	synaptopodin 2	6.31
Syt2	synaptotagmin II	5.72
Xlr	X-linked lymphocyte-regulated	5.68
Kcnd3	potassium voltage-gated channel, Shal-related family, member 3	5.62
Il1f5	interleukin 1 family, member 5 (delta)	5.61
Agfg2	ArfGAP with FG repeats 2	5.56
Ttc19	tetratricopeptide repeat domain 19	5.25
Cox7b2	cytochrome c oxidase subunit VIIb2	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
Psmd1	proteasome (prosome, macropain) 26S subunit, non-ATPase, 1	5.08
Bst2	bone marrow stromal cell antigen 2	4.96

Table2. Top 20 genes that were highly down-regulated in aged mouse hippocampus by 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	microRNA 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
Spta1	spectrin alpha, erythrocytic 1	-3.96
Cdv3	carnitine deficiency-associated gene expressed in ventricle 3	-3.89
Tmed7	transmembrane p24 trafficking protein 7	-3.79
AF366264	cDNA sequence AF366264	-3.70
Tsga10	testis specific 10	-3.68
Fcgr4	Fc receptor, IgG, low affinity IV	-3.53

Transcriptome analysis

Recent progress in genomics has enabled comprehensive analysis of cellular changes at the gene expression level. The DNA microarray technique has revealed various mechanisms of disease; however, no investigation of the association between POCD and the hippocampus has been done with a transcriptome-wide association study. Transcriptome analysis can capture both coding and non-coding RNA and quantify the heterogeneity of gene expression using samples from various tissues. This analysis has some advantages. Functional characterization and annotation of genes are possible, and researchers can reconstruct genetic interaction networks and identify clues for disease processes and prognoses.[35-38] In our experimental protocol, we performed whole transcriptome analysis to investigate the differences in gene expression in the hippocampus of mice between the Naïve group and the Ope group. Peffers et al. demonstrated drastic changes in RNA at numerous genomic levels, indicating that regulation of transcriptional networks is involved in aging-associated degeneration in a human clinical study.[39] Moreover, Broad et al. revealed that surgery-induced cell death is significantly increased in the developing piglet brain. Using RNA sequence data, this group identified changes in the expression of gene transcripts in isoflurane anesthesia alone compared to surgery.[40] These findings indicate that transcriptome analysis is useful for investigation of differences in gene expression before and after an intervention and can identify the association between these changes and the mechanism of the phenomenon. In our study, we detected up- and down-regulated genes that were affected by surgery. We also identified several target genes that may be related to cognitive deficits after the surgical procedure compared to before surgery, however, further experiments were needed to prove the role of gene. In this transcriptome analysis, the *Mapt* was an important target gene that was strongly associated with sevoflurane/surgery stress.

Mapt

Mapt is expressed in the central nervous system and encodes tau protein. Tau protein is necessary for maintaining axonal function, and it plays a role in axonal transport, synaptic plasticity, and nucleic acid protection.[41-46] However, once abnormal external stress and neuronal cell death occur in the brain, tau protein accumulates to form tauopathies. Tauopathies are a cause and pathogenic mechanism of neurodegenerative diseases, such as Alzheimer's disease, frontotemporal lobar degeneration, and post-traumatic brain injury.[47-50] The influence of tauopathies on cognitive function appears strong in aged individuals, and is the reason for the decrease in cerebral metabolism in elderly people. From the results of this study, we suggested two possibilities for the significant increase in the expression level of *Mapt*

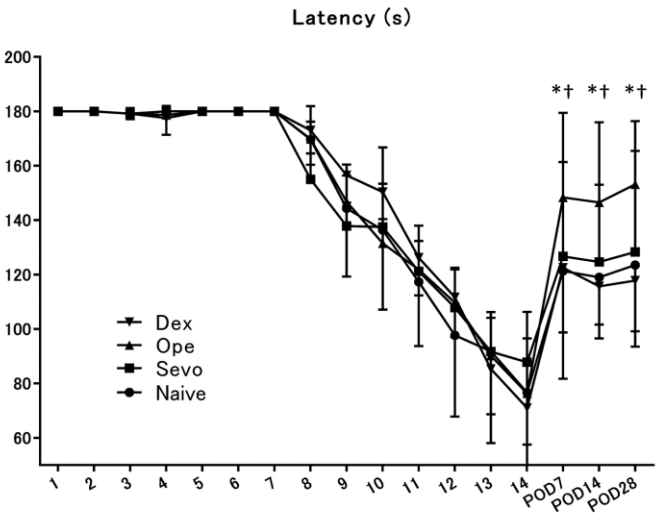

Fig. 4. Changes in latency (seconds) from the training to the test session. In training sessions over 14 days, the time to identify the target box (seconds) gradually decreased. On postoperative days 7, 14, and 28, the mice in the Ope group needed more time to find the hole compared to those in other groups. Dexmedetomidine alleviated the sevoflurane/surgery-induced cognitive deficit. The post hoc-adjusted p values is *p < 0.05 versus other groups. The time in the Ope group on postoperative days 7, 14, and 28 were significantly longer than those on training day 14 (†; p < 0.01).

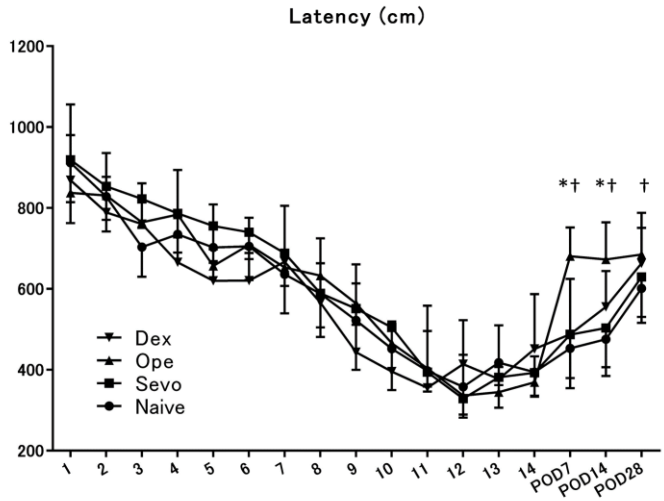

Fig. 5. Changes in latency (distance) from the training to the test session. In training sessions over 14 days, the distance to identify the target box (cm) gradually decreased. On postoperative days 7 and 14, the mice in the Ope group traveled a longer distance to find the hole compared to those in other groups. Dexmedetomidine alleviated the sevoflurane/surgery-induced cognitive deficit. The post hoc-adjusted p value is *p < 0.05 versus other groups. The distance in the Ope group on postoperative days 7, 14, and 28 were significantly longer than those on training day 14 (†; p < 0.01).

in the hippocampus of aged mice. *Mapt* may be directly increased as a consequence of the surgical procedure and/or *Mapt* may be indirectly increased due to suppression of the *Mapt* metabolism in the brain due to surgical stress. Inflammation caused by surgical stress results in neuroinflammation, neurodegeneration and neuronal cell death in the hippocampus. As a result, *Mapt* expression, which is thought to be involved in cognitive function, was drastically affected. This is a different process from the amyloid cascade observed in common neurodegenerative diseases. There are several reasons for up regulation in *Mapt*. It was suggested that there is a pathway that increases *Mapt* expression via up-regulating the gene associated with inflammation such as *Tmpo and Syt2*.

Moreover, the expression level of *Mapt* is associated with the pathogenesis of behavioral dysfunction and serves as a biomarker in the hippocampus of mice, indicating postoperative cognitive deficit. Dexmedetomidine treatment appeared to inhibit the production of the *Mapt* and improved the cognitive deficit.

In a surgical invasion model under general anesthesia, systemic inflammation spreads in the brain and, affects postoperative cognitive function. In our model, even short-term surgical invasion may lead to changes similar to age-related degeneration in the hippocampus.

Dexmedetomidine inhibits sevoflurane/surgery-induced up-regulation of Mapt gene expression

Dexmedetomidine, a selective α 2 adrenergic receptor agonist, is used clinically for perioperative sedation and anesthesia.[51,52] In addition, dexmedetomidine has anti-inflammatory actions by modulation of several pathways such as the cholinergic anti-inflammatory pathway and the NF-κB pathway, and this drug suppresses postoperative agitation and emergence delirium.[53,54] Systemic inflammation induced by LPS is suppressed by dexmedetomidine administration. Paeschke et al. investigated whether gene expression of interleukin-1β (IL-1β), tumor necrosis factor-α (TNF-α), and expression of the microRNAs miR 124, 132, 134, and 155 was changed in the adult rat hippocampus, cortex, and plasma after administration of 1 mg/kg LPS i.p. in the presence or absence of 5 μg/kg dexmedetomidine.[55] Dexmedetomidine suppressed LPS-induced neuroinflammation in the hippocampus and cortex via a significant reduction in IL-1β and TNF-α gene expression after 24 hours, and drastically decreased the expression of miR 124, 132, 134, and 155 after administration in both brain regions. Moreover, dexmedetomidine has a direct neuroprotective effect via induction of brain-derived neurotrophic factor (BDNF) and an indirect neuroprotective effect by promoting release of BDNF from astrocyte.[56]

In our study, we injected 10 μg/kg dexmedetomidine in the Dex group before the incision was made. All mice received a mild sedative without down saturation. In previous studies, the intensity of invasive stress and the amount of LPS administered were different, and the proper dose of dexmedetomidine also varied. Xiong et al. stated that 12 μg/kg dexmedetomidine is a high dose and that 3 μg/kg dexmedetomidine is a low dose.[57] Thus, the dose of dexmedetomidine that we used in our protocol (10μg/kg) was quite reasonable because mice were sedative without desaturation in this study. Even in a short-term surgical procedure, mRNA expression changes that are similar to changes due to aging likely occurred in the brain as a result of neuroinflammation. Moreover, dexmedetomidine attenuated up-regulation of the *Mapt* expression via several pathways and showed a neuroprotective effect. We observed only mRNA expression changes in the hippocampus and did not investigate pathways down-stream of mRNA or changes in protein expression. We focused on the *Mapt* in cognitive dificits, but other genes are likely involved as well. Indeed, the result of transcriptome analysis showed that many genes were up-regurated in this study. We could not determine whether direct reguration of *Mapt* via dexmedetomidine administration or not, and what gene was the main factor affected *Mapt* expression.

Dexmedetomidine attenuates sevoflurane/surgery-induced cognitive deficits

We demonstrated that dexmedetomoidine attenuated sevoflurane/surgery-induced cognitive deficits by down-regulating *Mapt* -mRNA expression. This result may explain one mechanism of the effect of dexmedetomidine and show a way to prevent cognitive deficits. To create the POCD model, mice aged 54-56 weeks underwent open abdominal surgery and intestinal manipulation. Results of the Barnes Maze test showed that the latency in the Ope group was significantly longer than that of naïve mice on 7, 14 and 28 day after surgery and that the distance in the Ope group was significantly longer than that of naïve mice on 7 and 14 day after surgery. Dexmedetomidine intervention before performing open abdominal surgery shortened the time latency in the Ope group on the 7, 14 and 28 day after surgery and the distance on the 7 and 14 day after surgery.

These results suggested that dexmedetomidine may be effective for improving the cognitive deficits induced by sevoflurane/surgery or for maintaining cognitive function after surgery stress. Xiong et al. also reported that dexmedetomidine may be able to limit the degree of expression of relaxin-3 and the over-expression of c-fos in the hippocampus, may down-regulate pro-apoptosis proteins such as Fas, caspase-8, and

caspase-9, and may up-regulate the anti-apoptosis protein Bcl-2.[55] Yamanaka et al. also showed that early dexmedetomidine treatment attenuates these systemically induced neurocognitive changes by preventing subsequent hippocampal neuroinflammation and overexpression of TLR-4 in microglia.[58] Our results were consistent with these previous results.

Limitations

In this experimental protocol, we observed relatively early behavioral changes at 7, 14, and 28 days after the intervention. In order to judge the behavioral disorder as POCD, we might need to observe longer periods. The analgesic effect of dexmedetomidine may have influenced behavioral changes after intervention. However, since both blood concentrations and tissue concentrations of dexmedetomidine were not examined in this experimental protocol, it is not possible to demonstrate the effect of dexmedetomidine on cognitive behavior after the intervention.

In the Dex group, the transcriptome analysis was not performed because the method of standard analysis in multiple groups has not been established. To clarify the mechanism of the effect of dexmedetomidine, it might be necessary to conduct a transcriptome analysis in the Dex group and to directly identify the differentially expressed genes.

In conclusion, dexmedetomidine attenuates sevoflurane/surgery-induced cognitive deficits and down-regulates *Mapt* expression in aged mice. Our findings suggest that have POCD may be improved by suppressing the production of *Mapt* expression.

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Conflict of Interest

The authors declare no competing interests**.**

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