Desflurane and Sevoflurane Induce Prolonged Motor Learning Deficits in aged App$^{NL-G-F/ NL-G-F}$ Mice.

Ryo Niikura
Yokohama City University School of Medicine Graduate School of Medicine: Yokohama Shiritsu Daigaku Igakubu Daigakuin Igaku Kenkyuka

Tomoyuki Miyazaki
Yokohama City University School of Medicine Graduate School of Medicine: Yokohama Shiritsu Daigaku Igakubu Daigakuin Igaku Kenkyuka

Kenkichi Takase (kenkichi.takase@jichi.ac.jp)
Jichi Medical University School of Medicine

Hiroki Sasaguri
Riken Center for Brain Science: RIKEN Noshinkei Kagaku Kenkyu Center

Takashi Saito
Riken Center for Brain Science: RIKEN Noshinkei Kagaku Kenkyu Center

Takaomi C Saido
Riken Center for Brain Science: RIKEN Noshinkei Kagaku Kenkyu Center

Takahisa Goto
Yokohama City University School of Medicine Graduate School of Medicine: Yokohama Shiritsu Daigaku Igakubu Daigakuin Igaku Kenkyuka

---

Research

Keywords: Alzheimer's disease, transgenic mice, general anesthesia, postanesthetic effects, behavioral phenotype, AppNL-G-F/ NL-G-F

Posted Date: December 10th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-122648/v1

Abstract

As the proportion of elderly in society increases, so does the number undergoing surgical procedures. The potential for anesthetics to facilitate Alzheimer's disease (AD) is an important issue. The causal relationship between clinical AD development and anesthesia remains conjectural, but preclinical studies have demonstrated that anesthesia, such as halothane, isoflurane and sevoflurane, but not desflurane, induce AD-like pathophysiological changes and cognitive impairments in transgenic mice AD models. Although it is expected desflurane will have more opportunities for use on elderly patients, little is known of its effects especially on non-cognitive functions such as motor and emotional functions. Thus, we examined the postanesthetic effect of desflurane and another common general anesthetic sevoflurane on motor and emotional function of aged $App^{NL\text{-}G\text{-}F/\ NL\text{-}G\text{-}F}$ (App-KI) mice. This is a recently developed transgenic mouse model of AD exhibiting amyloid b peptide (Ab) amyloidosis and neuroinflammatory response in age dependent manner without non-physiological amyloid precursor protein overexpression. Mice applied to a short test battery consisting of elevated plus maze, balance beam test, and tail suspension seven days after exposure to 8.0% desflurane for 6 hours or 2.8% sevoflurane for 2 h. App-KI mice showed significant increments in percentage of entry and time spent in open arms in elevated plus maze. Further, the number of slips and latency to traverse in balance beam test were also increased in App-KI mice. In tail suspension test, App-KI mice increased limb clasping score and immobile duration, but decreased the latency to first immobile episode compare to age-matched WT control. Desflurane and sevoflurane induced motor learning deficits in balance beam tests specifically in App-KI mice, while not affecting other behavioral performance. These results suggest high validity of App-KI mice as an animal model of AD. Use of anesthetics in AD patients and those at-risk requires careful and subsequent follow-up.

Introduction

Alzheimer's disease (AD) is the most common form of dementia and characterized by extracellular accumulation of amyloid β peptide (Aβ) accompany with neuroinflammation, intracellular neurofibrillary tangle caused by hyper-phosphorylation of tau protein, and loss of neuronal cells. Although main clinical symptoms are memory impairments and cognitive deficits, associated non-cognitive motor function and emotional disturbance have also been reported [1–3].

Aging is one of the major risk factors of AD, especially in the sporadic AD (SAD) which accounts for most cases. As the proportion of elderly in society increase, the number of AD and presumably presymptomatic AD patients undergoing surgery with general anesthesia may also increase. Thus, elucidating the causal relationships between general anesthesia and development of AD pathology is an urgent problem. Some retrospective cohort and case-control clinical studies suggested general anesthesia exposure associated with surgery was a risk factor for developing AD [4–7]. However other studies suggested no link between anesthesia/surgery and AD, dementia and cognitive dysfunctions [8–11]. In summary, whether anesthesia/surgery has causal links to developing AD remains conjectural [12–18].

Genetically modified mice are one of the most practical tools available today for translational AD research bridging clinical and basic researches. Many transgenic AD mouse models carry familial AD (FAD) mutations in genes that encode amyloid precursor protein (APP), presenilin-1 (PS1) and presenilin-2 (PS2). PS1 and PS2 are main components of γ-secretase that cleave APP to generate Aβ fragments. FAD accounts for less than 5% of all AD; however, causative genes have been identified, and it shows morphologically similar Aβ amyloidosis and tauopathy with SAD.

Studies have demonstrated exposure to general anesthesia such as halothane, isoflurane and sevoflurane contribute to cognitive impairment and exacerbate AD-like pathophysiological symptoms in transgenic AD mouse models [19–25]. Nevertheless, desflurane, another newer inhaled halogenated ether frequently used in clinical settings, has little effect on learning and memory of AD model mice. In fact, desflurane-treated 3xTgAD mice (i.e.. triple transgenic mice overexpressing APP with FAD Swedish mutation, mutant PS1 and human Tau transgenes), did not have cognitive impairment induced without surgical injuries [26]. Similarly, desflurane did not affect learning and memory performances in Barns maze of double transgenic mice carrying APP and PS1 transgenes with five FAD mutations, 5xFAD mice [27].

We recently first reported a comprehensive behavioral analysis of postanesthetic effects of desflurane on young adult mice [28], and demonstrated no observed differences between the control and desflurane-treated groups, excepted mild temporal effects on motor coordination in a balance beam test. In addition, desflurane-treated mice showed tendencies to increase time spent in open arms in the elevated plus maze, and to decrease latency for the first episode of immobility in tail suspension tests with sufficiently large effect size. These results suggested postanesthetic effects of desflurane mainly affect motor function and may also influence anxiety-like and depression-like behavior. The effects of desflurane on these functions may be more pronounced in fragile old animals or transgenic AD model mice, rather than in healthy young adults.

Another issue to consider is all previous studies investigating postanesthetic effects on transgenic AD model mice used transgenic mouse strains that overexpressed APP. Overexpression of APP induces Aβ overproduction causing insoluble Aβ aggregation, but simultaneously induces overproduction of other APP fragments with physiological effects unrelated to AD. $App^{NL-G-F/NL-G-F}$ (App-KI) mice were developed to address this issue and show increased Aβ42/40 ratio and Aβ deposition without non-physiological overexpression of APP. In App-KI mice, Aβ sequence was humanized and introduced three FAD mutations (i.e., Swedish, Beyreuther/Iberian and Arctic mutation) into the endogenous mouse App gene [29]. Further, App-KI mice showed Aβ amyloidosis, synaptic alterations and neuroinflammatory responses with slight memory impairment. Previous studies revealed various behavioral phenotypes of App-KI mice such as mild deficits in learning and memory function [29–35], sociality [30, 36] and anxiety [30, 32, 33, 36]. However, there are no studies focusing on the motor function of this App-KI mouse, as well as studies investigating the effect of general anesthesia on the motor function of this mouse line.

In our study, therefore, we investigated the effect of desflurane mainly on the motor function of aged App-KI mice and its age-matched controls, because it is expected that desflurane will have more opportunities to be used for elderly patients due to its beneficial properties, i.e. remaining in the body shorter than other inhaled halogenated anesthetics [37]. Furthermore, since sevoflurane is inhaled anesthesia frequently used in modern clinical settings as well as desflurane, we also assessed the effect of sevoflurane. We performed a short test battery composed of three behavioral tests, elevated plus maze, balance beam test, and tail suspension test, on mice exposed to desflurane and sevoflurane seven days prior to behavioral tests.

Methods

Animals

This study was carried out in strict accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the Yokohama City University. The protocol was approved by the Committee on the Ethics of Animal Experiments of the Yokohama City University [F-A-18-006, F-D-20-4].

App-KI mice that harbor Swedish and Beyreuther/Iberian mutations with the Arctic mutation in the App gene mice [29] and its wild type (C57BL/6J: WT) were used. The original lines of App-KI mice were obtained from RIKEN Center for Brain Science (Wako, Japan). Genotype was verified from tail biopsies by polymerase chain reaction (PCR). Detail protocol was described in Saito et al. (2014) [29]. Following cocktail of primers were used: 5'-ATCTCGGAAGTGAAGATG-3', 5'-ATCTCGGAAGTGAATCTA-3', 5'-TGTAGATGAGAACTTAAC-3' and 5'-CGTATAATGTATGCTATACGAAG-3'. App-KI and WT mice were housed in separate cages after 4 weeks of age. Mice were housed three to five per cage. Throughout the experiment, the animals were maintained in a temperature-controlled room (23 ± 2°C) with a 14-h light 10-h dark cycle (light period, 5:00 AM to 7:00 PM) and provided food and water *ad libitum*.

General anesthesia

When mice were 24 months old, App-KI and WT mice were randomly assigned to control group (APP-Air: N = 14, WT-Air: N = 7), desflurane exposure group (APP-Des: N = 12, WT-Des: N = 8) or sevoflurane exposure group (APP-Sev: N = 12, WT-Sev: N = 6). General anesthesia exposure was performed according to the method of our previous study [28, 38]. Briefly, mice undergoing general anesthesia were placed in a translucent plastic chamber (25.0 x 17.5 x 8.0 cm) within a thermostatic bath (34 ± 2°C). The chamber was maintained with oxygen and nitrogen (FiO_2 = 0.33) at 6 l/min. The concentration of desflurane and sevoflurane were kept at 8.0% for 6 h and 2.8% for 2 h respectively, corresponding to 1.3 minimum alveolar concentrations (MAC). The carbon dioxide in the chamber was maintained below 3 mmHg. The gases were monitored using a Capnomac ULTIMA monitor (Datex, Helsinki, Finland). After exposure, animals returned to their cages and fully recovered within 15 minutes. In the control group, one mouse at a time was placed in a plastic chamber flushed with the same carrier gas for 5 min and then returned to its original cage.

Behavioral test

One week after exposure to general anesthesia, the behavioral tests were serially carried out to assess the post-anesthetic effects of desflurane and sevoflurane on emotional and motor function of aged App-KI mice as outlined in Fig. 1. Before beginning testing, each mouse was observed in a clean cage for a general health check and neurological screening tests according to methods of our previous reports [28, 38-40]. The neurological screening tests were designed to detect any gross abnormalities in physical function. The ear-twitch reflex occurred when the pinna was touched with a cotton swab from behind, resulting in immediate movement of the ear. The eye-blink reflex occurred when a cotton swab was approaching the eye, resulting in blinking. The whisker-touch reflex was tested by lightly touching whiskers of a freely moving mouse. Normal mice will stop moving their whiskers and turn the head to the side on which the whiskers were touched. The postural reflex was evaluated by placing the mouse in an empty cage and shaking the cage, eliciting the extension of all four legs to keep an upright, balanced position. The righting reflex was tested by turning the mouse over onto its back, eliciting an immediate turnover response restoring upright posture on all four feet.

Elevated plus maze

To evaluate state anxiety-like behavior for open spaces and heights, elevated plus maze was conducted at day 1. The apparatus consisted of two open arms (297 x 54 mm) and two closed arms (300 x 60 x 150 mm) extending from a common central platform (60 x 60 mm). A small raised lip (3 mm) around the perimeter of the open arms prevented the mouse from falling. The apparatus was constructed from polypropylene, with gray floor and gray walls, and elevated 40 cm above the floor. Mice were placed individually on the center square facing an open arm and allowed to freely explore the apparatus under overhead fluorescent lighting (200 lx) for 5 min. Time spent in the open arms and open and closed arm entry (all four paws in an arm) were scored by a highly trained observer using behavioral scoring software (ANY-maze, Stoelting, IL, USA).

Balance-beam test

Motor coordination and balance were assessed by measuring ability of the mice to traverse a narrow beam to reach an enclosed safety platform at days 3. The beam consisted of long square strip of metal (1 m in length) with a cross-section of 12-mm. The beam was placed horizontally 50 cm above the bench surface, with one end mounted on a narrow support and the other end attached to an enclosed box (20 cm square) into which the mouse could escape. Lights (1200 lx) were positioned above and to one side of the start of the beam. Test was composed by 6 trials. In each trial, mice were placed at the start of the beam and allowed to traverse the beam to the enclosed box. The cut-off time of each trial was 60 s. The number of times the hind feet slipped off the beam, and latency to traverse the beam were recorded for each trial.

Tail suspension test

Postural reflex and coping behavior in a hopeless situation (antidepressant-like activity) were assessed by the tail suspension test at day 5. Mice were securely fastened to a flat metallic surface by the tip of the tail (2 to 3 cm) using medical adhesive tape and suspended 30 cm above the ground in a 40 cm^3 white Plexiglas box that isolated the mouse from visual distractions. The latency to first immobility defined as the absence of limb movement and the time of immobility were sampled by ANY-maze. Limb clasping behavior during the first 30s was monitored by trained experimenter and was scored on a scale from zero to four: 0 = no clasping behavior, 1 = one hind limb was retracted and the toes were splayed, 2 = both hind limbs were retracted and toes were splayed, 3 = both hind limbs were retracted and toes were clasping, 4 = clasping of all paws.

Statistical analysis

Data in elevated plus maze and latency to first immobility in tail suspension test were analyzed using a two-way analysis of variance (ANOVA) (Gene x Anesthesia). Data in balance beam test were analyzed using a three-way mixed designed ANOVA (Gene x Anesthesia x Trial) following by a post-hoc analysis adjusted by Shaffer's modified sequentially rejective Bonferroni procedure. Data in general health check, neurological screening tests and limb clasping in tail suspension test were analyzed using a Kruskal-Wallis H test following by a post-hoc analysis using Mann-Whitney U test adjusted by Bonferroni correction.

Results

General health

The general health check indicated no differences among all group [$p > .050$], except bald patch [$c^2(5) = 13.168$, $p = 0.022$] (Table 1). A post-hoc analysis revealed that App-KI mice showed more bald patches than WT control [$p = .001$]. The neurological reflexes of all groups showed similar performances [$p > .050$] (Table 1).

Elevated plus maze

The state anxiety-like behavior of mice was assessed by measuring percentage of open arm entries out of total arm entries and time spent in the open arms. The mean percentage of open arm entries out of total arm entries in the elevated plus maze was higher in the App-KI group compared to WT group [Main effect of Gene: $F(1, 53) = 17.017$, $p < .001$, partial $h^2 = .243$] (Fig. 2A). Percentage of open arm entries was not affected by general anesthesia exposure [Main effect of Anesthesia: $F(2, 53) = 1.657$, $p = .200$, partial $h^2 = .059$], and there was no interaction between Gene and Anesthesia [Interaction: $F(2, 53) = 0.609$, $p = .548$, partial $h^2 = .022$]. In the mean time spent in the open arm, the same tendency to percentage of open arm entries was observed [Main effect of Gene: $F(1, 53) = 28.159$, $p < .001$, partial $h^2 = .347$; Main effect of Anesthesia: $F(2, 53) = 0.493$, $p = .614$, partial $h^2 = .018$; Interaction: $F(2, 53) =$

0.135, p = .874, partial h^2 = .005] (Fig. 2B). These results suggested aged App-KI mice exhibited excessive anxiolytic-like behavior and both desflurane and sevoflurane did not affect these performances.

Balance beam test

Motor coordination and balance were assessed by measuring the latency to traverse and the number of times the hind feet slipped off in each trial of balance beam test. The mean number of times the hind feet slipped off of each group was shown in Fig. 3A, 3B, and 3C. A three-way repeated ANOVA revealed that the mean number of slips was larger in App-KI group compare to WT group [Main effect of Gene: F (1, 51) = 32.507, p < .001, partial h^2 = .389], and was decreased through the trials [Main effect of Trial: F (5, 255) = 14.328, p < .001, partial h^2 = .219]. General anesthetics did not affect the number of slips [Main effect of Anesthesia: F (2, 51) = 0.095, p = .909, partial h^2 = .004]. Although interactions between Gene and Anesthesia, Gene and Trial, and Anesthesia and Trial were not significant [p < .050], there was significant interaction between these three factors [F (10, 255) = 2.048, p = .041, partial h^2 = .074]. A post-hoc analysis revealed that in all trials, anesthetic exposure did not affect the number of slips in App-KI group or WT group [p > .050]. Further, the number of slips in the 1st and 2nd trial of the air-treated App-KI group were larger than that after third trial [ps < .050], suggesting that motor learning occurred in App-KI group when not exposed to anesthesia. On the other hand, in both App-KI groups exposed to anesthesia, the number of slips did not decrease with each trial [ps > .050]. In all the trials, excluding the 2nd trial of sevoflurane exposure groups, anesthesia-exposed App-KI groups exhibit more slips than anesthesia-exposed WT groups [ps < .050], whereas air-treated App-KI group showed more slips than air-treated WT group only in 1st and 2nd trials [p < .010], suggesting that both desflurane and sevoflurane induced motor learning deficits in App-KI mice.

The mean latency to traverse was longer in App-KI group compare to WT group [Main effect of Gene: F (1, 51) = 8.271, p = .006, partial h^2 = .140] (Fig. 3D, 3E, 3F). Similar to the number of slips, main effect of trial was significant [F (5, 255) = 46.216, p < .001, partial h^2 = .475], and main effect of anesthesia was not significant [F (5, 51) = 0.625, p < .539, partial h^2 = .024]. There was significant interaction between Gene and Trial [F (5, 255) = 3.945, p = .007, partial h^2 = .072], but other interactions were non-significant [p > .050]. A post-hoc analysis revealed the latency to traverse in APP-KI group was longer in 1st and 2nd trials compare to 3rd, 4th, 5th and 6th trials, while the latency in WT group was longer in 1st trial compare to the other trials, suggesting retarded motor learning in APP-KI mice.

Tail suspension test

To assess the postural reflex and antidepressant-like activity, limb clasping score, total immobile duration, and latency to first immobile episode were measured in the tail suspension test. A Kruskal-Wallis test revealed significant differences (c^2 (5) = 28.24, p < .001) in the limb clasping score (Fig. 4A). A post-hoc analysis revealed that App-KI group exhibit significant higher limb clasping score compared to WT group [U = 100, p < .001], whereas anesthesia did not affect limb clasping score [c^2 (2) = 0.44, p = .804]. The mean immobile duration in the tail suspension test was longer in the APP-KI group compared to WT

group [Main effect of Gene: $F(1, 50) = 11.773$, $p < .001$, partial $h^2 = .191$] (Fig. 4B). Immobile duration was not affected by general anesthesia exposure [Main effect of Anesthesia: $F(2, 50) = 0.508$, $p = .605$, partial $h^2 = .020$], and there was no interaction between Gene and Anesthesia [Interaction: $F(2, 50) = 1.210$, $p = .307$, partial $h^2 = .046$].

In the mean latency to first immobile episode in the tail suspension test, the same tendency to immobile duration was observed [Main effect of Gene: $F(1, 50) = 14.499$, $p < .001$, partial $h^2 = .225$; Main effect of Anesthesia: $F(2, 50) = 0.620$, $p = .542$, partial $h^2 = .024$; Interaction: $F(2, 50) = 0.160$, $p = .852$, partial $h^2 = .006$] (Fig. 4C). These results suggested aged App-KI mice exhibited deficits in posture reflex and earlier shift from active coping behavior to passive coping behavior, and both desflurane and sevoflurane did not affect these performances.

Discussion

In this study, we assessed effects of two types of inhaled anesthesia used in modern clinical settings - sevoflurane and desflurane- on anxiety, motor function, and depression of App-KI mice at an advanced age as a model for early-stage AD. Compared to age-matched WT controls, aged App-KI mice showed significant incremental increases in percentage of entry and time spent in open arms in elevated plus maze, in number of slips and latency to traverse in balance beam test, and in limb clasping score in tail suspension test. In the tail suspension test, increment in immobile duration and decrease in latency to first immobile episode were also observed. Neither desflurane nor sevoflurane affected behavioral performance of App-KI and WT mice except for motor learning performance of App-KI mice in the balance beam test.

In the elevated plus maze, App-KI mice showed increased anxiolytic-like behavior in comparison with WT mice. Our results are in line with previous studies that App-KI aged 3, 6–10 and 15–18 months [30, 33] exhibited anxiolytic-like behavior in elevated plus maze, as well as other several APP overexpressing animal model of AD [41–43], suggesting that the anxiolytic-like performance in these animals are associated with Aβ pathology rather than APP overexpression.

Motor dysfunction is an important phenotype of early AD that precedes classical cognitive impairment [1, 45, 46]; therefore, present results showing motor coordination in the balance beam test was impaired in App-KI mice is consistent with the fact that App-KI mice are an animal model mimicking feature of pre-clinical AD with mild decline in cognitive function accompanied by Aβ accumulation [29, 47]. Motoric deficits in App-KI mice might be attributed to changes of the pyramidal and extrapyramidal motor systems within the cerebellar cortex and spinal cord. Most currently available transgenic mouse models of AD, as well as human AD patients, showed progressive motor impairment with age [48–50]. Several of them identified that motor impairment in App mutants are due to an axonopathy in spinal cord motor neuron [51–53]. However, unlike App-KI mice used in the present study, all App mutants used in those studies shows non-physiological overexpression of APP, and such overexpression of APP results in axonal transport traffic jam [54] because APP per se associates with kinesin, a motor protein responsible

for anterograde axonal transport, via JIP-1 [55]. Such axonal blockage might cause intracellular accumulation of APP, rather than extracellular accumulation of Aβ, and induce axonal swellings. Interestingly, a recent study reported APP-overexpressing TgCRND8 mice showed motor deficits without axonopathy occurring in the brain and spinal cord [56], suggesting that deficits in axonal transport due to overproduction of APP itself causes motoric impairment. Since the App-KI mice show Aβ accumulation without non-physiological APP overexpression, motor dysfunction observed in this study may have derived from Aβ amyloidosis in the pyramidal and extrapyramidal motor system. However, effect of overproduced C-terminal fragment β of APP, because of increment in cleavage of APP by β-secretase derived from the Swedish mutation, on motor impairment cannot be ruled out.

In the present study, we demonstrated impairment in postural reflex of App-KI mice in the tail suspension test. Although deficits in postural reflex has been previously reported in Tg2576 mice [57] and TgCRND8 mice [58], the present study is the first report of postural reflex impairment in AD-model mice without APP overexpression. A longer immobile duration and shorter immobile latency observed in App-KI mice compare to WT mice were also observed. These results are typically interpreted as an increase in depression-like behavior. Similar to the postural reflex, although there are studies reported depression-like behavior in APP overexpressing mice [59, 60], this is the first study to demonstrate depression-like behavior in AD-model mice without APP overexpression. However, the fact that there is a possibility of contribution of motor deficit to the depressive-like behavior also should be carefully considered.

In the present study, we demonstrated that both desflurane and sevoflurane impaired motor learning of App-KI mice in a balance beam test, while air treated App-KI group exhibited improvement of performance. Since the cerebellum is important for procedural memory [61–64], it is likely that the motor learning deficits demonstrated in anesthesia-exposed App-KI mice are because of the pathophysiological changes in the cerebellum. In the animal model of AD, APP/PS1 mice and TgCRND8 mice at preplaque stage showed motor deficits and decline in long-term depression (LTD) in cerebellar parallel fiber-Purkinje cell synapse, suggesting impairment in neurobiological basis of short-term motor learning [58, 65]. Furthermore, the cerebellum, especially Purkinje cells, is vulnerable organ for various toxic damage [66, 67] including general anesthesia such as sevoflurane [68] and desflurane [69]. Taken together, the cerebellum is a vulnerable region to Aβ pathology, and present study suggested that general anesthesia might have potent to exacerbate such pathological changes. We recently reported that desflurane induced no postanesthetic effects in healthy young adult mice excepted mild temporal effects on motor coordination, suggesting safety of desflurane in clinical settings [28]. In contrast, anesthesia-induced motor learning deficits in the App-KI mice in the present study indicated that anesthetic must be used carefully in case of patients with AD-like pathological alteration.

Elderly people with cognitive impairment, especially patients with AD, are potentially at a higher risk of falling compare to age-matched healthy control [70, 71]. Deficit in higher-level cognitive functions such as executive dysfunction and anosognosia are also risk factors for falling as well as age related physical decline, such as sensory deficit and loss in muscle strength [72]. Furthermore, in the prospective cohort study, Stark et al. (2013) [73] demonstrated that presumptive preclinical AD without cognitive

impairments showed higher falling probability. These results are compatible with findings in previous and present studies demonstrating motor impairment in animal model of pre-clinical AD [1, 44–46, 51–53]. Furthermore, anesthesia-induced prolonged deficit in motor learning in App-KI mice shown in this study suggested that use of anesthetics in AD patients and or those at-risk requires careful and subsequent follow-up.

There are two major limitations in this study that could be addressed in future research. First, although the App-KI mice is one of the most practical animal models of AD at present, they do not fully replicate human AD pathology. They do not exhibit tauopathy, neuronal loss and severe behavioral phenotypes [29]. In addition, this animal has been genetically modified for Aβ production, aggregation, and degradation, but remains unclear about Aβ clearance. Further genetic manipulation will be required. Second, in this study, we assessed only three behavioral tests. In order to describe a more accurate behavioral phenotype, it is necessary to evaluate them from multiple perspectives including cognitive and other non-cognitive functions. Moreover, the underlying mechanisms of postanesthetic effects on motor learning deficits specific to App-KI mice remains unclear due to the lack of physiological indicators.

In conclusion, desflurane and sevoflurane induced motor learning deficits in App-KI mice, but not in WT control. However, further studies are required to reveal neurophysiological basis of effects of anesthesia on motor learning in AD model animals. Nevertheless, this is the first report to assess the effect of general anesthesia on motor learning in App-KI mice exposed to desflurane and sevoflurane.

Abbreviations

Alzheimer's disease
AD
myloid β peptide
Aβ
sporadic Alzheimer's disease
SAD
familial Alzheimer's disease
FAD
$App^{NL-G-F/NL-G-F}$
App-KI
wild type
WT
polymerase chain reaction
PCR
minimum alveolar concentrations
MAC
analysis of variance
ANOVA

long-term depression
LTD

Declarations

Authors' contributions

RN, TM, and KT conceived and performed the behavioral experiments, and wrote the manuscript. HS, TS, and TCS generated APP-KI mice and wrote the manuscript. TG provided expertise and feedback, and secured funding. All authors read and approved the final manuscript.

Acknowledgements

The authors thank Prof. Adam Jon Lebowitz for English proofreading.

Ethics approval

All of the experiments involving mice were conducted using protocols approved by the Institutional Animal Care and Use Committee of Yokohama City University.

Consent for publication

All of the authors have given their consent for publication.

Availability of data and materials

The datasets supporting the conclusions of this article are included within the manuscript.

Competing interests

The authors declare no competing interest.

Funding

This work was supported by JSPS KAKENHI Grant Number 16K15678 to G.T.

Authors' information (optional)

Correspondence should be addressed to Tomoyuki Miyazaki and Kenkichi Takase.

Ryo Niikura and Tomoyuki Miyazaki contributed equally to this work.

Department of Anesthesiology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Ryo Niikura

Department of Anesthesiology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Department of Physiology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Tomoyuki Miyazaki

Laboratory of Psychology, Jichi Medical University School of Medicine, Tochigi, Japan

Kenkichi Takase

Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science, Wako, Japan

Hiroki Sasaguri

Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science, Wako, Japan

Department of Neurocognitive Science, Nagoya City University Graduate School of Medical Sciences, Nagoya, Japan

Takashi Saito,

Laboratory for Proteolytic Neuroscience, RIKEN Center for Brain Science, Wako, Japan

Takaomi C. Saido

Department of Anesthesiology, Yokohama City University Graduate School of Medicine, Yokohama, Japan

Takahisa Goto

References

1. Buchman AS, Bennett DA. Loss of motor function in preclinical Alzheimer's disease. Expert Rev Neurother. 2011;11(5):665–76.
2. Lyketsos CG, Carrillo MC, Ryan JM, Khachaturian AS, Trzepacz P, Amatniek J, Cedarbaum J, Brashear R, Miller DS. Neuropsychiatric symptoms in Alzheimer's disease. Alzheimers Dement. 2011;7(5):532–9.
3. Zhao QF, Tan L, Wang HF, Jiang T, Tan MS, Tan L, Xu W, Li JQ, Wang J, Lai TJ, Yu JT. The prevalence of neuropsychiatric symptoms in Alzheimer's disease: Systematic review and meta-analysis. J Affect Disord. 2016;190:264–71.
4. Bohnen N, Warner MA, Kokmen E, Kurland LT. Early and midlife exposure to anesthesia and age of onset of Alzheimer's disease. Int J Neurosci. 1994;77(3–4):181–5.
5. Chen CW, Lin CC, Chen KB, Kuo YC, Li CY, Chung CJ. Increased risk of dementia in people with previous exposure to general anesthesia: a nationwide population-based case-control study. Alzheimers Dement. 2014;10(2):196–204.

6. Chen PL, Yang CW, Tseng YK, Sun WZ, Wang JL, Wang SJ, Oyang YJ, Fuh JL. Risk of dementia after anaesthesia and surgery. Br J Psychiatry. 2014;204(3):188–93.
7. Lee JJ, Choi GJ, Kang H, Baek CW, Jung YH, Shin HY, Park YH, Woo YC. Relationship between Surgery under General Anesthesia and the Development of Dementia: A Systematic Review and Meta-Analysis. Biomed Res Int. 2020;2020:3234013.
8. Aiello Bowles EJ, Larson EB, Pong RP, Walker RL, Anderson ML, Yu O, Gray SL, Crane PK, Dublin S. Anesthesia Exposure and Risk of Dementia and Alzheimer's Disease: A Prospective Study. J Am Geriatr Soc. 2016;64(3):602–7.
9. Avidan MS, Searleman AC, Storandt M, Barnett K, Vannucci A, Saager L, Xiong C, Grant EA, Kaiser D, Morris JC, Evers AS. Long-term cognitive decline in older subjects was not attributable to noncardiac surgery or major illness. Anesthesiology. 2009;111(5):964–70.
10. Gasparini M, Vanacore N, Schiaffini C, Brusa L, Panella M, Talarico G, Bruno G, Meco G, Lenzi GL. A case-control study on Alzheimer's disease and exposure to anesthesia. Neurol Sci. 2002;23(1):11–4.
11. Sprung J, Warner DO, Knopman DS, Petersen RC, Mielke MM, Jack CR Jr, Lowe VJ, Martin DP, Hanson AC, Schroeder DR, Przybelski SA, Schulte PJ, Weingarten TN, Vemuri P. Exposure to surgery with general anaesthesia during adult life is not associated with increased brain amyloid deposition in older adults. Br J Anaesth. 2020;124(5):594–602.
12. Avidan MS, Evers AS. Review of clinical evidence for persistent cognitive decline or incident dementia attributable to surgery or general anesthesia. J Alzheimers Dis. 2011;24(2):201–16.
13. Baranov D, Bickler PE, Crosby GJ, Culley DJ, Eckenhoff MF, Eckenhoff RG, Hogan KJ, Jevtovic-Todorovic V, Palotás A, Perouansky M, Planel E, Silverstein JH, Wei H, Whittington RA, Xie Z, Zuo Z. Consensus statement: First International Workshop on Anesthetics and Alzheimer's disease. Anesth Analg. 2009;108(5):1627–1630.
14. Bittner EA, Yue Y, Xie Z. Brief review: anesthetic neurotoxicity in the elderly, cognitive dysfunction and Alzheimer's disease. Can J Anaesth. 2011;58(2):216–23.
15. Jiang J, Jiang H. Effect of the inhaled anesthetics isoflurane, sevoflurane and desflurane on the neuropathogenesis of Alzheimer's disease (review). Mol Med Rep. 2015;12(1):3–12.
16. Seitz DP, Shah PS, Herrmann N, Beyene J, Siddiqui N. Exposure to general anesthesia and risk of Alzheimer's disease: a systematic review and meta-analysis. BMC Geriatr. 2011;11:83.
17. Vutskits L, Xie Z. Lasting impact of general anaesthesia on the brain: mechanisms and relevance. Nat Rev Neurosci. 2016;17(11):705–17.
18. Wu L, Zhao H, Weng H, Ma D. Lasting effects of general anesthetics on the brain in the young and elderly: "mixed picture" of neurotoxicity, neuroprotection and cognitive impairment. J Anesth. 2019;33(2):321–35.
19. Bianchi SL, Tran T, Liu C, Lin S, Li Y, Keller JM, Eckenhoff RG, Eckenhoff MF. Brain and behavior changes in 12-month-old Tg2576 and nontransgenic mice exposed to anesthetics. Neurobiol Aging. 2008;29(7):1002–10.

20. Feng C, Liu Y, Yuan Y, Cui W, Zheng F, Ma Y, Piao M. Isoflurane anesthesia exacerbates learning and memory impairment in zinc-deficient APP/PS1 transgenic mice. Neuropharmacology. 2016;111:119–29.
21. Geng P, Zhang J, Dai W, Han X, Tan Q, Cheng D, Fang P, Liu X. Autophagic Degradation Deficit Involved in Sevoflurane-Induced Amyloid Pathology and Spatial Learning Impairment in APP/PS1 Transgenic Mice. Front Cell Neurosci. 2018;12:185.
22. Jia Z, Geng L, Xie G, Chu Q, Zhang W. Sevoflurane impairs acquisition learning and memory function in transgenic mice model of Alzheimer's disease by induction of hippocampal neuron apoptosis. Int J Clin Exp Med. 2015;8(9):15490–7.
23. Li C, Liu S, Xing Y, Tao F. The role of hippocampal tau protein phosphorylation in isoflurane-induced cognitive dysfunction in transgenic APP695 mice. Anesth Analg. 2014;119(2):413–9.
24. Perucho J, Rubio I, Casarejos MJ, Gomez A, Rodriguez-Navarro JA, Solano RM, De Yébenes JG, Mena MA. Anesthesia with isoflurane increases amyloid pathology in mice models of Alzheimer's disease. J Alzheimers Dis. 2010;19(4):1245–57.
25. Perucho J, Casarejos MJ, Gomez A, Solano RM, de Yébenes JG, Mena MA. Trehalose protects from aggravation of amyloid pathology induced by isoflurane anesthesia in APP(swe) mutant mice. Curr Alzheimer Res. 2012;9(3):334–43.
26. Tang JX, Mardini F, Janik LS, Garrity ST, Li RQ, Bachlani G, Eckenhoff RG, Eckenhoff MF. Modulation of murine Alzheimer pathogenesis and behavior by surgery. Ann Surg. 2013;257(3):439–48.
27. Miao H, Dong Y, Zhang Y, Zheng H, Shen Y, Crosby G, Culley DJ, Marcantonio ER, Xie Z. Anesthetic isoflurane or desflurane plus surgery differently affects cognitive function in Alzheimer's disease transgenic mice. Mol Neurobiol. 2018;55(7):5623–38.
28. Niikura R, Miyazaki T, Yonezaki K, Uchimoto K, Takase K, Goto T. Exploratory analyses of postanesthetic effects of desflurane using behavioral test battery of mice. Behav Pharmacol. 2020;31(7):597–609.
29. Saito T, Matsuba Y, Mihira N, Takano J, Nilsson P, Itohara S, Iwata N, Saido TC. Single App knock-in mouse models of Alzheimer's disease. Nat Neurosci. 2014;17(5):661–3.
30. Latif-Hernandez A, Shah D, Craessaerts K, Saido T, Saito T, De Strooper B, Van der Linden A, D'Hooge R. Subtle behavioral changes and increased prefrontal-hippocampal network synchronicity in APP^{NL-G-F} mice before prominent plaque deposition. Behav Brain Res. 2019;364:431–41.
31. Masuda A, Kobayashi Y, Kogo N, Saito T, Saido TC, Itohara S. Cognitive deficits in single App knock-in mouse models. Neurobiol Learn Mem. 2016;135:73–82.
32. Mehla J, Lacoursiere SG, Lapointe V, McNaughton BL, Sutherland RJ, McDonald RJ, Mohajerani MH. Age-dependent behavioral and biochemical characterization of single APP knock-in mouse ($APP^{NL-G-F/NL-G-F}$) model of Alzheimer's disease. Neurobiol Aging. 2019;75:25–37.
33. Sakakibara Y, Sekiya M, Saito T, Saido TC, Iijima KM. Cognitive and emotional alterations in App knock-in mouse models of Aβ amyloidosis. BMC Neurosci. 2018;19(1):46.

34. Sakakibara Y, Sekiya M, Saito T, Saido TC, Iijima KM. Amyloid-β plaque formation and reactive gliosis are required for induction of cognitive deficits in App knock-in mouse models of Alzheimer's disease. BMC Neurosci. 2019;20(1):13.
35. Whyte LS, Hemsley KM, Lau AA, Hassiotis S, Saito T, Saido TC, Hopwood JJ, Sargeant TJ. Reduction in open field activity in the absence of memory deficits in the App^{NL-G-F} knock-in mouse model of Alzheimer's disease. Behav Brain Res. 2018;336:177–81.
36. Pervolaraki E, Hall SP, Foresteire D, Saito T, Saido TC, Whittington MA, Lever C, Dachtler J. Insoluble Aβ overexpression in an App knock-in mouse model alters microstructure and gamma oscillations in the prefrontal cortex, affecting anxiety-related behaviours. Dis Model Mech. 2019;12(9):dmm040550.
37. Eger EI, Eisenkrafr JB, Weiskopf RB. The Pharmacology of Inhaled Anesthetics. Chicago: HealthCare Press; 2002.
38. Yonezaki K, Uchimoto K, Miyazaki T, Asakura A, Kobayashi A, Takase K, Goto T. Postanesthetic effects of isoflurane on behavioral phenotypes of adult male C57BL/6J mice. PLoS One. 2015;10(3):e0122118.
39. Takase K, Tsuneoka Y, Oda S, Kuroda M, Funato H. High-fat diet feeding alters olfactory-, social-, and reward-related behaviors of mice independent of obesity. Obesity (Silver Spring). 2016;24(4):886–94.
40. Kikuchi M, Takase K, Hayakawa M, Hayakawa H, Tominaga SI, Ohmori T. Altered behavior in mice overexpressing soluble ST2. Mol Brain. 2020;13(1):74.
41. Escrig A, Canal C, Sanchis P, Fernández-Gayol O, Montilla A, Comes G, Molinero A, Giralt M, Giménez-Llort L, Becker-Pauly C, Rose-John S, Hidalgo J. IL-6 trans-signaling in the brain influences the behavioral and physio-pathological phenotype of the Tg2576 and 3xTgAD mouse models of Alzheimer's disease. Brain Behav Immun. 2019;82:145–59.
42. Lalonde R, Fukuchi K, Strazielle C. APP transgenic mice for modelling behavioural and psychological symptoms of dementia (BPSD). Neurosci Biobehav Rev. 2012;36(5):1357–75.
43. Ognibene E, Middei S, Daniele S, Adriani W, Ghirardi O, Caprioli A, Laviola G. Aspects of spatial memory and behavioral disinhibition in Tg2576 transgenic mice as a model of Alzheimer's disease. Behav Brain Res. 2005;156(2):225–32.
44. O'Keeffe ST, Kazeem H, Philpott RM, Playfer JR, Gosney M, Lye M. Gait disturbance in Alzheimer's disease: a clinical study. Age Ageing. 1996;25(4):313–6.
45. Scarmeas N, Hadjigeorgiou GM, Papadimitriou A, Dubois B, Sarazin M, Brandt J, Albert M, Marder K, Bell K, Honig LS, Wegesin D, Stern Y. Motor signs during the course of Alzheimer disease. Neurology. 2004;63(6):975–82.
46. Waite LM, Grayson DA, Piguet O, Creasey H, Bennett HP, Broe GA. Gait slowing as a predictor of incident dementia: 6-year longitudinal data from the Sydney Older Persons Study. J Neurol Sci. 2005;229–230:89–93.
47. Sasaguri H, Nilsson P, Hashimoto S, Nagata K, Saito T, De Strooper B, Hardy J, Vassar R, Winblad B, Saido TC. APP mouse models for Alzheimer's disease preclinical studies. EMBO J. 2017;36(17):2473–87.

48. Lalonde R, Fukuchi K, Strazielle C. Neurologic and motor dysfunctions in APP transgenic mice. Rev Neurosci. 2012;23(4):363–79.
49. Wirths O, Weis J, Szczygielski J, Multhaup G, Bayer TA. Axonopathy in an APP/PS1 transgenic mouse model of Alzheimer's disease. Acta Neuropathol. 2006;111(4):312–9.
50. Wirths O, Breyhan H, Schäfer S, Roth C, Bayer TA. Deficits in working memory and motor performance in the APP/PS1ki mouse model for Alzheimer's disease. Neurobiol Aging. 2008;29(6):891–901.
51. Cotel MC, Jawhar S, Christensen DZ, Bayer TA, Wirths O. Environmental enrichment fails to rescue working memory deficits, neuron loss, and neurogenesis in APP/PS1KI mice. Neurobiol Aging. 2012;33(1):96–107.
52. Jawhar S, Trawicka A, Jenneckens C, Bayer TA, Wirths O. Motor deficits, neuron loss, and reduced anxiety coinciding with axonal degeneration and intraneuronal Aβ aggregation in the 5XFAD mouse model of Alzheimer's disease. Neurobiol Aging. 2012;33(1):196.e29-40.
53. Stokin GB, Lillo C, Falzone TL, Brusch RG, Rockenstein E, Mount SL, Raman R, Davies P, Masliah E, Williams DS, Goldstein LS. Axonopathy and transport deficits early in the pathogenesis of Alzheimer's disease. Science. 2005;307(5713):1282–8.
54. Gunawardena S, Goldstein LS. Disruption of axonal transport and neuronal viability by amyloid precursor protein mutations in Drosophila. Neuron. 2001;32(3):389–401.
55. Chiba K, Chien KY, Sobu Y, Hata S, Kato S, Nakaya T, Okada Y, Nairn AC, Kinjo M, Taru H, Wang R, Suzuki T. Phosphorylation of KLC1 modifies interaction with JIP1 and abolishes the enhanced fast velocity of APP transport by kinesin-1. Mol Biol Cell. 2017;28(26):3857–69.
56. Yuan Q, Yang J, Wu W, Lin ZX. Motor deficits are independent of axonopathy in an Alzheimer's disease mouse model of TgCRND8 mice. Oncotarget. 2017;8(58):97900–12.
57. Seo JS, Leem YH, Lee KW, Kim SW, Lee JK, Han PL. Severe motor neuron degeneration in the spinal cord of the Tg2576 mouse model of Alzheimer disease. J Alzheimers Dis. 2010;21(1):263–76.
58. Russo R, Cattaneo F, Lippiello P, Cristiano C, Zurlo F, Castaldo M, Irace C, Borsello T, Santamaria R, Ammendola R, Calignano A, Miniaci MC. Motor coordination and synaptic plasticity deficits are associated with increased cerebellar activity of NADPH oxidase, CAMKII, and PKC at preplaque stage in the TgCRND8 mouse model of Alzheimer's disease. Neurobiol Aging. 2018;68:123–33.
59. Francis BM, Yang J, Hajderi E, Brown ME, Michalski B, McLaurin J, Fahnestock M, Mount HT. Reduced tissue levels of noradrenaline are associated with behavioral phenotypes of the TgCRND8 mouse model of Alzheimer's disease. Neuropsychopharmacology. 2012;37(8):1934–44.
60. Romano A, Pace L, Tempesta B, Lavecchia AM, Macheda T, Bedse G, Petrella A, Cifani C, Serviddio G, Vendemiale G, Gaetani S, Cassano T. Depressive-like behavior is paired to monoaminergic alteration in a murine model of Alzheimer's disease. Int J Neuropsychopharmacol. 2014;18(4):pyu020.
61. Boyden ES, Katoh A, Raymond JL. Cerebellum-dependent learning: the role of multiple plasticity mechanisms. Annu Rev Neurosci. 2004;27:581–609.
62. De Zeeuw CI, Hoebeek FE, Bosman LW, Schonewille M, Witter L, Koekkoek SK. Spatiotemporal firing patterns in the cerebellum. Nat Rev Neurosci. 2011;12(6):327–44.

63. Ito M. Long-term depression. Annu Rev Neurosci. 1989;12:85–102.
64. Ito M. Mechanisms of motor learning in the cerebellum. Brain Res. 2000;886(1–2):237–45.
65. Kuwabara Y, Ishizeki M, Watamura N, Toba J, Yoshii A, Inoue T, Ohshima T. Impairments of long-term depression induction and motor coordination precede Aβ accumulation in the cerebellum of APPswe/PS1dE9 double transgenic mice. J Neurochem. 2014;130(3):432–43.
66. Fonnum F, Lock EA. Cerebellum as a target for toxic substances. Toxicol Lett. 2000;112–113:9–16.
67. Manto M, Oulad Ben Taib N. The contributions of the cerebellum in sensorimotor control: what are the prevailing opinions which will guide forthcoming studies? Cerebellum. 2013;12(3):313–5.
68. Yukina GY, Sukhorukova EG, Belozertseva IV, Polushin YS, Tomson VV, Polushin AY. Cerebellar Cortex Neurons and Microglia Reaction to Sevoflurane Anesthesia. Cell tissue biol. 2019;13:439–45.
69. Mapelli J, Gandolfi D, Giuliani E, Prencipe FP, Pellati F, Barbieri A, D'Angelo E, Bigiani A. The effect of desflurane on neuronal communication at a central synapse. PLoS One. 2015;10(4):e0123534.
70. Morris JC, Rubin EH, Morris EJ, Mandel SA. Senile dementia of the Alzheimer's type: an important risk factor for serious falls. J Gerontol. 1987;42(4):412–7.
71. Sheridan PL, Hausdorff JM. The role of higher-level cognitive function in gait: executive dysfunction contributes to fall risk in Alzheimer's disease. Dement Geriatr Cogn Disord. 2007;24(2):125–37.
72. Muir SW, Gopaul K, Montero Odasso MM. The role of cognitive impairment in fall risk among older adults: a systematic review and meta-analysis. Age Ageing. 2012;41(3):299–308.
73. Stark SL, Roe CM, Grant EA, Hollingsworth H, Benzinger TL, Fagan AM, Buckles VD, Morris JC. Preclinical Alzheimer disease and risk of falls. Neurology. 2013;81(5):437–43.

Tables

Due to technical limitations, table 1 is only available as a download in the Supplemental Files section.

Figures

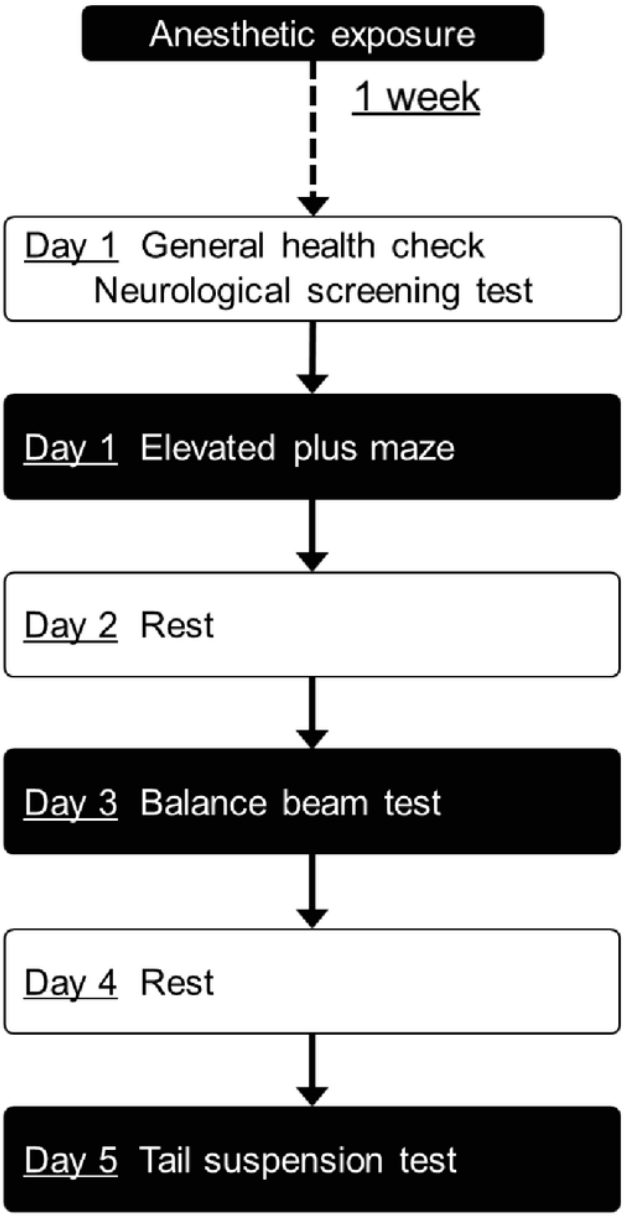

Figure 1

Timeline of the series of behavioral tests One week after exposure to general anesthesia, the behavioral tests were serially carried out. After general health check and neurological screening tests, mice were examined an elevated plus maze on day 1. After one day of rest to avoid the carry-over effect, a balance beam test was performed on day 3 and as well, a tail suspension test was performed on day 5.

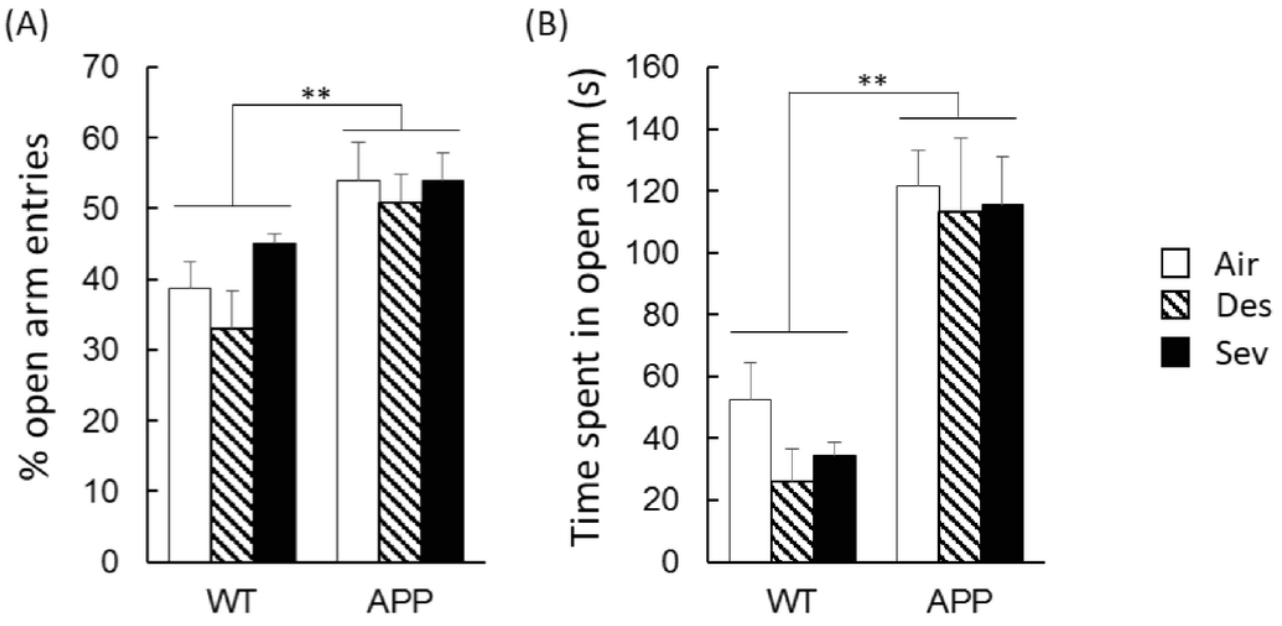

Figure 2

Effect of general anesthesia on aged APP-KI mice in an elevated plus maze Mean (+ SEM) percentage of open arm entries out of total arm entries (A) and time spent in open arm (B) in each group, WT-Air (n = 7), WT-Des (n = 8), WT-Sev (n = 6), APP-Air (n = 14), APP-Des (n = 12), or APP-Sev (n = 12) group, are shown. *p < .050; **p < .010

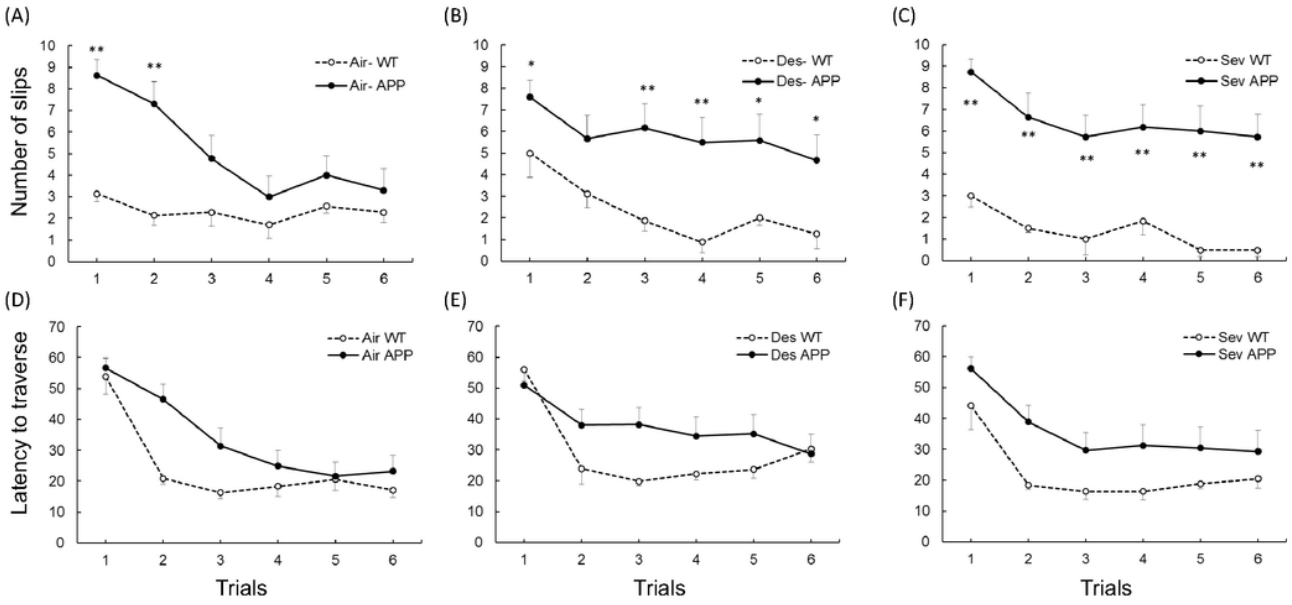

Figure 3

Effect of general anesthesia on aged APP-KI mice in a balance beam test Mean (+ SEM) number of times the hind feet slipped off in each trial (A, B, C) and latency to traverse (D, E, F) in each group, WT-Air (n = 7), APP-Air (n = 14), WT-Des (n = 8), APP-Des (n = 12), WT-Sev (n = 6), or APP-Sev (n = 11) group, are shown. *p < .050; **p < .010 compared to corresponding WT group

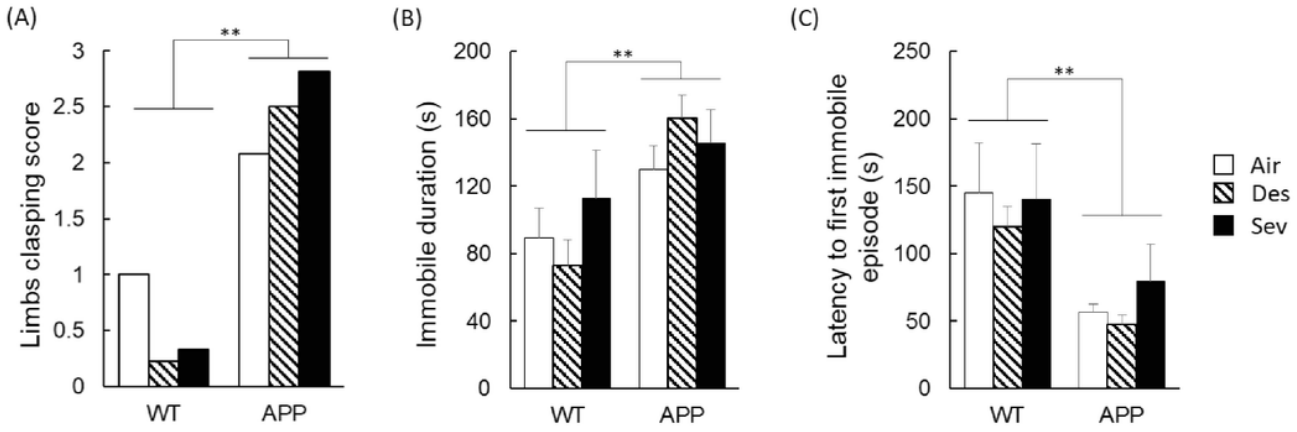

Figure 4

Effect of general anesthesia on aged APP-KI mice in a tail suspension test Mean (+ SEM) limbs clasping score (A), immobile duration (B) and latency to first immobile episode (C) in each group, WT-Air (n = 7), WT-Des (n = 8), WT-Sev (n = 6), APP-Air (n = 13), APP-Des (n = 11), or APP-Sev (n = 11) group, are shown. **p < .010

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- Table.1.xlsx