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# Effects of propofol and surgery on neuropathology and cognition in the 3xTgAD Alzheimer transgenic mouse model

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## Abstract

**Background.** Previous work suggests that anaesthesia and surgery amplify the pathology and cognitive impairment of animals made vulnerable via age or specific transgenes. We hypothesized that surgery under propofol anaesthesia, a widely used i.v. general anaesthetic, has minimal delayed cognitive and neuroinflammatory sequelae in a vulnerable mouse transgenic model.

**Methods.** We conducted caecal ligation and excision surgery in cognitively presymptomatic (11-month-old) 3xTgAD mice under i.p. propofol anaesthesia. Age-matched 3xTgAD control mice received vehicle or propofol without surgery. Morris water maze testing was conducted 3 and 15 weeks later. Brains were examined with quantitative immunohistochemistry for amyloid β plaques, tau pathology, and microglial activation. Acute changes in neuroinflammatory cytokines were assessed in separate cohorts at 6 h.

**Results.** We detected no significant differences between groups in escape latencies at either 3 or 15 weeks, but detected a significant effect of surgery in the probe test at both 3 and 15 weeks. Spatial working memory was unaffected at 16 weeks in any group. No effects of either propofol alone or propofol with surgery were detected on plaque formation, tau aggregates, or neuroinflammation. Acute biochemical assays detected no effects in brain interleukin-10 or interleukin-6 concentrations.

**Conclusions.** Surgery in a vulnerable transgenic mouse under propofol anaesthesia was associated with minimal to no changes in short- and long-term behaviour and no changes in neuropathology. This suggests that propofol anaesthesia is associated with better cognitive outcomes in the aged, vulnerable brain compared with inhalation anaesthesia.

**Key words**: Alzheimer's disease; anaesthetics; neurogenic inflammation; postoperative complications

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**Editor's key points**

- Surgery and anaesthesia have been implicated in producing postoperative cognitive dysfunction in the vulnerable brain.
- Propofol alone or propofol with surgery had minimal effects on cognitive function and neuropathological markers in a mouse model of Alzheimer's disease.
- Given previous data that show impaired cognitive function after surgery and inhalation anaesthesia, these findings suggest that surgery with propofol results in better cognitive outcomes in the vulnerable brain.

Elderly patients frequently report cognitive decline after surgery, a complaint known as postoperative cognitive dysfunction (POCD), a label that is currently undergoing re-evaluation.[1] Thus, interest in the relationship between anaesthetics, surgery, postoperative dementia, and Alzheimer's disease has increased. Certain anaesthetics alone increase the production[2] and aggregation[3 4] of amyloid β protein and detach and aggregate tau.[5–7] Moreover, in Alzheimer mouse models, exposure to volatile anaesthetics produces an acceleration of both amyloidopathy[8 9] and tauopathy.[10] However, only transient cognitive dysfunction follows volatile anaesthetic exposures in both older wild-type mice[8] and rats,[11 12] and cognitive effects were undetected 3 months after anaesthesia alone in even the triple transgenic Alzheimer mouse model (3xTgAD).[10]

Anaesthesia is typically accompanied by surgery, and surgery can have independent effects on inflammation and cognition. Cognitive dysfunction occurs after tibial surgery in young-adult wild-type mice, resolving by 7 days, and in aged rats with a minor procedure up to 3 weeks after surgery.[13 14] However, cognitive impairment up to at least 3 months after abdominal surgery under desflurane anaesthesia was reported in the 3xTgAD mouse model.[15] Propofol, a common i.v. general anaesthetic, is known to have anti-inflammatory properties. We hypothesized that the neuroinflammation associated with surgery, leading to accelerated Alzheimer's neuropathology and symptoms, is reduced by propofol compared with volatile anaesthesia. We test this hypothesis in the setting of a vulnerable brain: the 3xTgAD mouse model.

# Methods

## Animals

The University of Pennsylvania institutional animal care and use committee approved the protocol; mice were treated in accordance with American Physiological Society and National Institutes of Health guidelines, and the ARRIVE (Animal Research: Reporting of In Vivo Experiments) Guidelines[16] were followed for animal reporting. Mice were bred and housed in a University of Pennsylvania Animal facility under a 12 h–12 h light–dark cycle, with room temperature and humidity constantly maintained, and all cages contained nestlets. Homozygous 3xTgAD mice,[17] harbouring the PS1M146V, APPSwe, and tau P301L transgenes, were bred using initial breeding pairs courtesy of Dr Frank LaFerla at University of California Irvine, CA, USA. Genotype was verified from tail biopsies by standard approaches. Microchips (BioMedic Data Systems, Inc., Seaford, DE, USA) were implanted s.c. at 3 weeks of age for identification and to blind the experimenter. The age range was 9–14 months (11 months mean age) at the time of anaesthesia and surgery. At this age, 3xTgAD mice have underlying progressive Alzheimer's neuropathology, but are cognitively presymptomatic, which mimics older surgical patients. The study was designed to determine whether surgery and anaesthesia accelerate the onset of underlying disease markers and result in long-term cognitive dysfunction. Male and female 3xTgAD mice were randomly divided into three experimental conditions as outlined below. Sample sizes were chosen based on our previous study[15] and others,[18 19] where statistically significant effects in 11-month-old 3xTgAD mice have been detected with the Morris water maze (MWM) using six to 12 animals per group. A total of 55 3xTgAD mice were used in this study. Wild-type controls were not repeated because no surgical effect was found in our previous study.[15]

## Control and anaesthesia-alone groups

### *Vehicle control group*

Fourteen control mice were injected with 10% Liposyn II (Hospira, Lake Forest, IL, USA) at 12.5 ml $kg^{-1}$ and returned to their home cage. For the acute biochemical assays $n$=5, and for the first behavioural time point $n$=9. One control mouse died during behavioural testing; thus $n$=8 controls for behavioural studies.

### *Anaesthesia-alone group*

Pilot studies determined that emulsified propofol 250 mg $kg^{-1}$ i.p. provided 20–30 min of surgical anaesthesia. We solubilized neat propofol at 20 mg $ml^{-1}$ into 10% Liposyn II (Hospira) rather than using commercial material. Twenty mice were given one injection of propofol (250 mg $kg^{-1}$), placed in a heated and humidified chamber flushed continuously with 30% oxygen (balance nitrogen), and continuously observed for breathing, heart rate, and skin colour. Three of 20 mice died immediately after propofol injection i.p., probably as a result of vascular injection. Thus, $n$=5 for the acute biochemical studies and $n$=12 for the anaesthesia-only behavioural studies.

## Surgery and anaesthesia group

Anaesthesia was induced as in control animals, but the mice were given 30% oxygen (balanced with nitrogen) via nose cone for surgery. The caecal ligation and excision (not puncture) was performed in aseptic conditions by the same technician, as previously reported.[15] For postoperative pain, we injected bupivacaine 0.25% (10 mg $kg^{-1}$ s.c.) in the incision margins. Mice also received Primaxin (imipenem and cilastatin sodium) (Merck & Co, Inc, Whitehouse Station, NJ, USA), (20 mg $kg^{-1}$) in 1 ml of normal saline s.c. Weights and rectal temperature were recorded before and after surgery. The average duration of surgery was 18 min (range 15–25 min); average duration of anaesthesia was 25 min (range 20–35 min). On completion of surgery, animals were placed in a warmed container and observed for several hours before returning to their home cage. Mice in the acute group were killed 6 h after anaesthesia or anaesthesia with surgery. Twenty-one mice were included in the surgery group; two mice died immediately after propofol i.p., one was euthanized 24 h after surgery, one was killed before the first MWM session, and two died between MWM sessions. Final groups were $n$=5 for the acute cytokine studies, $n$=12 for the first MWM testing, and $n$=10 for the second MWM session.