Published in final edited form as:

Ann Surg. 2013 March ; 257(3): 439–448. doi:10.1097/SLA.0b013e318269d623.

Modulation of murine Alzheimer pathogenesis and behavior by surgery

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Abstract

Objective—Previous research suggests a link between anesthetic exposure and Alzheimer’s disease exists. Since anesthetics are rarely given alone, we ask whether addition of surgery further modulates Alzheimer’s disease.

Background—Cognitive dysfunction occurs after surgery in humans. Anesthesia alone produces cognitive decline in both older wild-type mice and rats, and the addition of surgery produces transient decline in young adult wild type mice. Because neuroinflammation has been implicated, and occurs early in Alzheimer’s disease, we hypothesize that the neuroinflammatory stress associated with surgery will accelerate the progression of Alzheimer’s disease.

Methods—Cecal ligation and excision was performed on pre-symptomatic 5–11 month-old triple-transgenic Alzheimer (3xTgAD) and C57BL/6 wild type mice under desflurane anesthesia. Surgery animals were compared to aged matched 3xTgAD and WT mice exposed to air or desflurane alone. Cognitive function was assessed via Morris water maze at 2 and 13 weeks post-operatively. Amyloid and tau pathology, as well as inflammation and synaptic markers were quantified with immunohistochemistry, Luminex, ELISA or western blot assays.

Results—A significant cognitive impairment in 3xTgAD mice that underwent surgery compared with air or desflurane controls, persisted to at least 14 weeks after surgery. Microglial activation, amyloidopathy and tauopathy were enhanced by surgery as compared to desflurane alone. No differences between surgery, anesthetic or air controls were detected in WT mice

Conclusions—Surgery causes a durable increment in Alzheimer pathogenesis, primarily through a transient activation of neuroinflammation.

INTRODUCTION

Age is a risk factor for both AD and for needing surgery. Over 200 million surgical procedures occur worldwide¹ and about half of all people will have anesthesia and surgery in their lifetime. Thus, the significance of an interaction between anesthesia, surgery and Alzheimer’s disease is of interest, but clinical studies are inconclusive. Certainly the

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Conflict of interest: All authors declare no conflicts of interest.

existence of short term (up to 3 months) post-operative cognitive decline (POCD) is well documented in elderly patients^{2,3}. Further, three independent studies showed that the age of Alzheimer's disease diagnosis is associated with previous anesthetic exposure at odds ratios between 1.2 – 1.7^{4–6}. However, a more recent study of an Alzheimer Disease Center cohort concluded that the trajectory of cognitive decline was unaltered by either surgery or major illness⁷. Shortly after this article appeared, a larger study reported that major illness was associated with cognitive decline in the elderly following hospitalization⁸. Given the effect sizes that would be considered important, all of the reported clinical studies must be considered underpowered, and thus the translational question remains unanswered.

More progress has been made in animals, although ambiguity still exists. Anesthetics alone have been found to increase the production^{9,10} and aggregation of amyloid beta protein¹¹, central features of at least some forms of Alzheimer's disease. Exposure to anesthetics alone in two Alzheimer mouse models^{12,13} has also revealed an acceleration of amyloidopathy without enhanced cognitive dysfunction. An acceleration of tau neuropathology has also been found after both hypothermic and normothermic¹⁴ anesthetic exposures in young adult wild type mice¹⁵, in the 3xTgAD mouse model after normothermic anesthetic exposures¹⁶, and in lipopolysaccharide induced neuroinflammation¹⁷. Transient cognitive dysfunction has been reported following anesthetic exposures in both older wild-type mice¹² and rats^{18,19}. Cognitive effects, however, have not always been observed after anesthesia alone, especially in wild-type and/or young rodents^{16,20,21}. Long-term cognitive decline (>3 months) after anesthesia, has not been reported in the rodent.

Since volatile anesthetics are rarely given in the absence of surgery, it is possible that the surgical procedure itself modulates the progression of Alzheimer pathology. The peripheral surgical stress response, as described in detail by Kohl and Deutschman²², is a complex process involving many distinct alterations of the normal physiology, including production of inflammatory mediators, increase in catabolism of skeletal and visceral muscle, increased gluconeogenesis, and increased protein synthesis. In at least one study, cognitive decline and microglial activation was observed following tibial surgery-induced systemic inflammation in wild type mice²³. Because neuroinflammation occurs early in the pathology in 3xTgAD mice²⁴, we hypothesized that the significant physiologic and neuroinflammatory stress associated with surgery accelerates the progression of Alzheimer's pathology and consequent cognitive decline beyond that caused by the anesthetic exposure.

METHODS

Animals

Animal protocols were approved as required by the University of Pennsylvania Institutional Animal Care and Use Committees and all mice were treated in strict accordance to APS/NIH guidelines. Homozygote triple transgenic Alzheimer (3xTgAD) mice, harboring the presenilin -1 (PS1^{M146V}), amyloid precursor protein (APP^{Swe}), and tau_{p301L} transgenes, were bred using an initial 3xTgAD breeding pair courtesy of Dr. Frank LaFerla at UC Irvine, CA and elderly C57BL/6 wild type (WT) mice were obtained from the National Institute of Aging. The 3xTgAD mouse model has been shown to develop both plaque and neurofibrillary tangle neuropathology and cognitive dysfunction in an age-dependent manner²⁵. Genotype was verified from tail biopsies by means of proteinase-K digestion, DNA extraction and PCR. Mice were tagged with BMDS IMI-1000 subcutaneous identification tags (BioMedic Data Systems, Seaford, DE) at weaning. Both male and female mice were used in this study, and the age range of 5–11 months was deliberately chosen to represent a cross section of older but still pre-symptomatic animals that are highly reflective of the corresponding ages of patients presenting for surgery.

Surgery and anesthetic exposure

Three experimental conditions were examined: desflurane only (Des), desflurane plus surgery (Surg) and air controls (Con). Gender and ages of each group of mice were carefully matched to minimize confounding effects of either.

Desflurane only—3xTgAD and WT mice were exposed to desflurane (2,2,2-trifluoro-1-fluoroethyl-difluoromethyl ether) alone, with no surgery. Desflurane was chosen because it is a commonly used anesthetic in the elderly and is considered less provocative than isoflurane or halothane^{26,27}, thereby improving the odds of detecting an effect due to surgery. Exposure was conducted in a heated, monitored anesthesia chamber, with humidified 40% O₂ balanced by N₂, initially containing 1.5 MAC (minimum alveolar concentration) (11% v/v) desflurane and 100% oxygen flowing at 5L/min, using a calibrated desflurane vaporizer. Desflurane concentration was reduced to 9% (v/v) for maintenance after loss of response to foot pinch while maintaining spontaneous respiration. Anesthetic concentrations were monitored during exposures with a Datex Engstrom Capnomac gas monitor. The duration of the exposure was less than 30 min, and the mice resumed full activity within 10 min after exposure. Chamber CO₂ levels never exceeded 0.1%. Rectal temperatures were monitored before and periodically during the anesthetic exposures and up to 72 h post-operative (PO) and changes great than 1 °C were not detected. Body weights were recorded prior to and after exposure which remained unchanged.

Desflurane and Surgery—3xTgAD and WT mice underwent desflurane anesthesia as described above. After induction, mice were transferred to a heated table where they were induced and maintained as above (average 8.75% desflurane) with 100% oxygen at 1 L/min via a nose cone. The abdomen was shaved, and cleaned with Betadine swabs. Using sterile technique, a laparotomy incision of about 1.5cm in length, extending from just below the xiphoid process to the just above the pubic symphysis was made. The bowel was exteriorized and the cecum was identified. Using 4.0 coated, braided silk suture, the cecum was ligated within 1cm of the distal ileum, using care to avoid obstruction. The cecum was then excised just distal to the ligation, and the remaining proximal tip of cecum was copiously irrigated with 10cc of normal saline to remove any fecal material. The bowel was then returned to the abdominal cavity, and the abdomen closed in two layers using 4.0 coated, braided suture. A subcutaneous injection of 0.25% Bupivacaine (10mg/kg) was given at the wound edges, as well as a subcutaneous injection of 1cc of normal saline for hydration. Body weights and temperatures were recorded as above, with no significant changes detected. The average duration of the surgery was 19 min (range 14–24 min), and the average duration of the anesthetic exposure was 24 min (range 19–30 min). All mice were ambulatory within 30 min following surgery and had returned to apparently normal activity within 24h. Surgical wounds healed well with no drainage, odor, or erythema. The activity of all mice prior to behavioral training and testing was considered normal.

Controls—Groups of comparably aged 3xTgAD and WT mice exposed to humidified 30% O₂ (balanced by N₂) in other ongoing studies were used to collect control behavioral data.

Behavioral testing

Morris water maze—Learning and memory testing was conducted using the Morris Water Maze (MWM) as described previously¹⁶. Briefly, at both time periods (Fig. 1), the mice were first trained to escape from the pool (4 60s trials per day for 5 days), followed by the behavioral testing, including reference memory testing (4 60s trials per day for 5 days) and a probe test (one 60s trial). In addition, spatial working memory testing was conducted at the second time point (up to 8 trials per day for 10 days). Water temperature was maintained at 23–26°C with a pond heater and there was a 30–45 min interval between

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trials. Mice were dried off and actively warmed after each trial. Cued training (prior to the cognitive function behavioral tests) trained the mice to reach a flagged submerged platform to be removed from the pool. All mice learned to escape from the flagged platform. After training, reference memory testing was conducted at 2 and 13 wk PO. This tested the ability of the mice to learn the location of the hidden platform in a fixed location from varied starting points by using fixed external visual cues in the room. Mice were tracked with a video camera mounted above the pool that fed to a computer running IMAQ PCI-1407 software (National Instruments, Austin TX). The mean escape latencies were calculated for each mouse per day. In addition, the distance traveled was recorded for each mouse during the reference memory trials and the data expressed as the mean distance traveled for each day. The platform was removed for the probe test which examined the ability of mice to remember the location of the platform by measuring preference for the quadrant where the platform had been located (target quadrant) over the other quadrants. The probe trial was conducted 2 h after the final trial on the last day of the reference memory testing for each mouse (Fig. 1). The percent time spent in each quadrant, during the 60s trial, was recorded and the ratios of percent time in the target quadrant to the percent time in the opposite quadrant were calculated for each animal and the data expressed as the mean of these ratios. Swim speed during the probe trial was also measured. Beginning at 12 wk PO, the same behavioral tests were repeated for the same animals (Fig.1). In addition, spatial working memory was conducted beginning at 14 wk PO as described previously^{16,28}. The criterion for this test involved locating different (pre-set) locations of the hidden platform three times in a row within 20s by using external visual cues in the room. The number of trials to reach criterion for each mouse for each platform location was determined and the mean number of trials to reach criterion per platform location was calculated.

Rotorod—Approximately 24h after completing the MWM tasks, Rotorod® trials were conducted to assess motor and coordination effects of the surgery and desflurane groups, about 16 weeks PO. The test was conducted in two parts. Initially, each mouse was acclimated to the Rotorod® (IITC Series 8, Life Sciences) with a 60 s trial at 9 rpm. After the training trial, a series of three test trials were conducted at 18rpm. For the test trials, the length of time the mice remained on the rod up to a maximum of 120 s was recorded. When the mouse fell off the rod, the time was recorded and that test trial ended. There was a 15 min break between each of the three test trials. Three trials were conducted and mean times determined.

Statistics—All behavioral analyses were performed using GraphPad Prism 5.04 software and animal numbers are described in the legend for Figures 2 and 3 and Supplemental Digital Content 1.

Euthanasia and tissue collection—Animals that underwent behavioral studies were euthanized the day after the Rotorod® testing by rapid induction of deep anesthesia with halothane and then intracardiac perfusion and simultaneous exsanguination from the right atrium with ice cold phosphate buffered saline. The time required from loss of consciousness to brain removal was routinely less than 5 min., and we have never been able to document differences in brain biochemistry as compared to more conventional methods of euthanasia such as carbon dioxide inhalation or cervical dislocation (unpublished results). The brains were placed on ice and hemisected in the sagittal plane and one side was placed in 4% paraformaldehyde and subsequently paraffin embedded while the other side was immediately frozen in liquid nitrogen and stored at -80°C for biochemical analyses.

Immunohistochemistry and biochemistry measurements

Amyloid and tau pathology, as well as inflammatory and synaptic markers were quantified by immunohistochemistry, Luminex or western blot assays. In addition to the mice that underwent long-term behavioral analyses, the acute response to desflurane and surgery was assessed in additional 3xTgAD and WT mice at 5–11 months of age which were euthanized 24h after surgery as above for immunohistochemical and biochemical measurements.

Immunohistochemistry—Immunohistochemistry was performed essentially as described previously¹⁶. Paraffin embedded brain sections were rehydrated and incubated with primary antibody 6E10 (1:1000; Covance, Dedham, MA) for amyloid plaque, antibody AT8 (1:500; Thermo Scientific, Waltham, MA) specific for PHF-tau doubly phosphorylated at Ser201 and Thr205, and Iba-1 antibody (1:1000; Wako Pure Chemical Industries, Japan) for activated microglia. Immunostaining was visualized using an Olympus IX70 Microscope equipped with a SensiCam SVGA High-Speed Cooled Digital Camera (Cooke Corp., Auburn Hills, MI) and images of the hippocampal CA1 region and subiculum were captured at 10X magnification. The number and area occupied by plaque and tau stained cells and the area occupied by microglial cells were quantified using IPLab Suite v4.0 Imaging Processing and Analysis software (Biovision Technologies, Exton, PA) and the mean calculated from three slides per animal.

Luminex Assay—Determination of acute cytokines and chemokines levels was conducted with the Luminex assay in Human Immunology Core at the University of Pennsylvania and as described²⁹ using frozen saline perfused homogenized 3xTgAD and WT hemibrains at 24 h PO. Concentrations of IL-1 β , IL-4, IL-6, IL-10, TNF- α and MIG were simultaneously quantified for each sample using Mouse Cytokines/Chemokines Panel-5 Plex (Millipore catalog# MPXMCYTO-70K). In addition, using the Luminex xMAP platform, the concentration of TGF- β in brain was measured with the Milliplex MAP TGF β 1-Single Plex (Millipore, catalog #TGFB-64K-01). The data were expressed as the ratio to wild type controls.

Western blot assays—Frozen saline perfused hemibrains from 24h PO 3xTgAD and WT mice were homogenized in 2% SDS or NP40 Cell Lysis Buffer (Invitrogen catalog# FNN0021) supplemented with 1mM PMSF (Sigma catalog#P7626) and protease inhibitor cocktail (Sigma catalog# P-2714). Immunoblot assays were performed essentially as described previously¹⁶ for APP, CTFs, caspase-3, synaptophysin, PSD-95, and Iba-1.

Statistical analyses—Statistics were performed for genotype and treatment using one-way or two-way ANOVAs, as described in the figure legends. Animal numbers are indicated in the legends for Figures 4, 5 and 6. All statistics were done using GraphPad Prism 5.04 software.

RESULTS

Behavior

Escape latency—The escape latencies showed no changes at either 2 wk or 13 wk post-operative (PO) in the wild type mice (Fig 2A, C) but a significant treatment effect was found in the 3xTgAD surgical mice compared to air controls at both 2 wk PO (Fig 2B) and 13 wk PO (Fig 2D). The desflurane group was not significantly different from controls in either WT or 3xTgAD groups at either time point, although there was a trend in the 3xTgAD group at 2 wk PO. The mean path lengths for the 5 days of reference memory testing was determined and no differences were found in the wild type groups (Fig 2 E,G) but significantly increased path lengths were observed for the 3xTgAD surgical mice compared

to controls at both 2 and 13 wk PO (Fig 2 F,H). Path length, unlike escape latency, is independent of swim speed. Both of these analyses indicate that the surgical 3xTgAD mice were cognitively compromised at both 2 wk PO and 3 months PO, despite having been trained prior to testing at both time points.

Probe test—A significant transgene effect was found at the 2-week time point for the probe test (Fig. 3A,B), and a treatment effect was found for the 13 wk PO probe test, with the 3xTgAD surgical group performing significantly poorer than their controls (Fig. 3B). This significant effect for the surgical 3xTgAD mice is also found when all quadrants were compared (See Figure, Supplemental Digital Content 1, with the probe test data expressed as percent time in each quadrant).

Spatial working memory—The number of trials to reach criterion per platform location was determined beginning at 14 wk PO, with a lower number of trials reflecting better performance. The 3xTgAD mice that underwent surgery performed significantly less well than the air controls (Fig. 3C). No significant differences were found in the WT groups. The 3xTgAD air controls performed as well as the WT air controls.

Motor Skills—During the 60 s probe test, swim speed was also recorded (Fig. 3D, E), with significant differences for both WT and 3xTgAD anesthetic and surgery mice compared to controls. Rotorod® testing at 16 wk PO revealed no differences in motor or coordination ability between the surgery and desflurane-only treated animals (Fig. 3F).

Immunohistochemistry (24h & 16wk PO)

Amyloid β and tau—After completion of the behavioral testing (16wk PO), euthanasia and brain harvest, amyloid β plaque density was quantified in the subiculum in 3xTgAD mice with immunohistochemical localization of 6E10 (Fig. 4 A–C). Phosphorylated tau was detected with the AT8 antibody (Fig. 4D–F). Only a non-significant trend for higher plaque density in the surgery group as compared to the desflurane group was noted, (Fig. 4C). On the other hand, a highly significant increase in the number of pTau positive neurons was found in the 3xTgAD surgery group, with the greatest numbers of tau aggregates found in the subiculum (Fig. 4H). WT mice were not examined since amyloid plaque and pTau (human isotope) are generally not detectable.

Activated microglia—The number of activated microglia at 24h and 16wk PO were quantitated with Iba-1 immunohistochemistry in the CA1 region and the underlying molecular layer of the dentate gyrus of the hippocampus in both WT (Fig. 5A, B, C) and 3xTgAD mice (Fig. 5 D, E, F). At 24h PO, a significant increase in activated microglia was found in the surgery groups in both 3xTgAD and WT mice, with the largest increase observed in the Alzheimer transgenic mice (Fig. 5G). However, at 16wk PO, no differences in microglia were detected in any group (Fig. 5H).

Biochemistry

Luminex assays—At 24h PO, brain cytokine levels in 3xTgAD and WT mice were measured with the Luminex assay. Brains had been cold-saline perfused at euthanasia to minimize cytokine contamination arising from blood. Significant treatment, genotype and interaction effects were found with interleukin-10 (IL-10) (Fig. 6A). IL-10 was increased after desflurane or desflurane and surgery in WT mice, but only in the 3xTgAD animals that were exposed to desflurane. IL-10 was unaltered in the 3xTgAD animals treated with both desflurane and surgery, perhaps reflecting unopposed pro-inflammatory effects in the transgenic brain after surgery. No significant changes were found for transforming growth factor beta (TGF- β), interleukin-1 beta (IL-1 β), or tumor necrosis factor alpha (TNF- α)

(Fig. 6B–D). A significant treatment and genotype effect were found for the chemokine, Monokine induced by IFN-gamma (MIG) (Fig. 6E). IL-4 and IL-6 were undetectable with Luminex assays in the brain at 24h PO.

Western immunoblots—Significant differences were not detected in 3xTgAD and WT whole brain homogenates for amyloid precursor protein (APP), C-terminal fragments (CTFs), caspase-3, synaptophysin, post-synaptic density-95 (PSD-95), and the microglial marker, Iba-1, at 24 h PO (See Figure, Supplemental Digital Content 2, for the Western blot results.) This may be due to the use of whole brain homogenates, and thus dilution of regional effects.

DISCUSSION

The cognitive battery used in this study clearly revealed a persistent decrement in learning and memory, caused by the addition of an abdominal surgical procedure to a brief inhalational anesthetic in the context of the brain made vulnerable by human Alzheimer-associated transgenes. The surgical procedure roughly mimics an appendectomy or colectomy, exceedingly common procedures received by millions of patients annually. There are two important points to consider regarding this result. First, at the time of surgery and even 3 months later, this control 3xTgAD animal was essentially asymptomatic; its baseline learning and memory were not different from the WT animals despite having evidence of ongoing neuropathology. This is likely to well represent both the age range and cognitive status of many of our patients presenting for a surgical procedure, and suggests the window of vulnerability extends into this presymptomatic period. Previous work in a slightly different murine Alzheimer model (Tg2576) suggested that vulnerability to interventions might be lost when the animals demonstrate significant cognitive impairment¹², termed a “floor effect”, although surgery was not part of the intervention. Second, the WT animal demonstrated no significant cognitive sequelae from either the anesthetic or the surgery. This is in contrast to previous work where surgery caused a pronounced transient cognitive decline in WT rodents, with complete resolution of the cognitive deficits by post-operative day 7^{23,30}. Although the surgical procedures were different, the likely explanation for our inability to see an effect on the WT animal is time. We did not begin testing until 14 days after surgery in order to assure that animals were well beyond the pain and recovery phase of the surgery. Thus, it is possible that our WT animals also suffered a transient cognitive decline in the first week after surgery, but our hypothesis was focused more on the long term effects.

The basis for slower swim speed in the surgical groups is not clear, as the animals had no signs of pain or altered movement, and were feeding and grooming normally. It is possible that this observation confounded the behavioral tests, specifically the escape latency, a time, and therefore, swim-speed dependent task. However, several observations argue against this. First, the WT mice had the same decrement in swim speed (Fig 3D,E), but no alteration in escape latency at either 2 or 13 weeks post-operation (Fig 2A,C). Second, the same task can be analyzed in a swim-speed *independent* manner by measuring the escape path length, which was also significantly elevated in the surgery group (Fig 2F,H). Third, another MWM swim-speed *independent* task, the probe test, was definitive in its demonstration of impaired memory in the transgenic surgery mice (Fig 3B). Finally, the Rotorod® test, a test of both coordination and motor function, was unaltered in the surgery mice (Fig 3F). Taken together, these four observations effectively rule out the contribution of swim speed alterations to the deficits attributed to learning and memory.

The brain vulnerability studied here may not translate completely to the human situation. The 3xTgAD model, like all current murine models of Alzheimer’s disease, more closely

resembles the situation in familial Alzheimer's (an overproduction of aggregating protein species), which constitute only a small minority of Alzheimer patients. Thus, it is not yet clear whether results from this kind of model well represent most of our patients who have, or will eventually get, late-onset (sporadic) Alzheimer's disease. However, these models are currently the standard of choice for screening new Alzheimer drugs, and have yielded considerable insight into Alzheimer pathogenesis. Further, the 3xTgAD animal most closely recapitulates the essential neuropathological hallmarks of even late-onset Alzheimer's; extracellular senile plaque, intracellular neurofibrillary tangles and synaptic dysfunction²⁵. Finally, the order of progression of the pathology is consistent with human late-onset disease. Thus, although triggering details of the model imperfectly represent most human Alzheimer's disease, the general features suggest translatability.

The underlying mechanism for the cognitive effects observed here appears to be triggered by peripheral inflammation, consistent with previous studies that show lipopolysaccharide injection enhances both neuroinflammation, cognitive decline and Alzheimer pathogenesis in several transgenic mouse models {1977, 1615, 2092}. In this study, in both the middle-aged WT and 3xTgAD animals, the addition of surgery caused an early increase in Iba-1 immunoreactivity in the brain. This is probably due to microglial activation, although attraction of other cell types (monocytes) to the brain, via a leaky blood brain barrier, cannot be fully ruled out. Regardless, this suggests an inflammatory process which is well-known to occur as a result of surgery, at least in the periphery²². It is also well-known that this peripheral inflammatory response, at least as marked by cytokines, is dampened in the brain, perhaps explaining our inability to detect significant changes in brain tissue. It might be important to note that we carefully cleared the brain of blood during euthanasia in order to exclude the possibility of cytokine/chemokine contamination from blood, which is known to be very rich in these molecules after surgery²². This initial acute inflammatory response is clearly not sustained. The differential Iba-1 immunoreactivity is absent at 16 weeks post-operatively, suggesting a return to baseline conditions. It is possible that whole brain homogenates obscured regional influences, but despite this, the effect on the ongoing Alzheimer pathogenesis is indicated by the pronounced difference on tau phosphorylation, and the suggestion of increases in amyloid β plaque.

In previous studies, we and others have demonstrated that the anesthesia alone caused a delayed cognitive decline and/or a change in neuropathology in Alzheimer mice^{12,16} and in aged WT rats^{18,19,31} and mice¹². In contrast, in this study, the anesthetic alone here was not associated with a significant change in cognition or neuropathology. The likely reasons are twofold. First, the previous studies used either isoflurane; isoflurane with nitrous oxide, or halothane, the former of which has been implicated in a wide variety of neurological insults³². Halothane is no longer used because of its greater metabolic breakdown and association with hepatotoxicity. Desflurane, although understudied in this context, has been less associated with cognitive problems or neurotoxicity, and therefore a preferable drug for revealing an increment due to surgery. Desflurane is very commonly used for surgical anesthesia in elderly patients. The second major difference between this and previous studies is the duration of exposure. We only kept the animal anesthetized for as long as it took to perform the surgery, never more than 30 min. Previous work has suggested that neurotoxicity is a function of both concentration and duration of exposure³². Thus, the lack of significant effects of the anesthetic here should not necessarily be viewed as exonerating desflurane. It is possible that significant effects would emerge with longer exposures, as suggested by the trend for worse cognition at 2 weeks after exposure to desflurane alone.

A syndrome called "post-operative cognitive dysfunction", or POCD, has been coined to refer to the commonly reported loss of cognitive abilities, usually in the elderly, in the days to weeks following surgery. Much studied, POCD has not been convincingly demonstrated

to persist after 3 months in people, thus it appears to be either reversible, or in some manner compensated for. However, in this study, the cognitive deficits after surgery were essentially unchanged after 4 months in the 3xTgAD mouse, suggesting a lasting and perhaps irreversible effect. Thus, these results suggest that the interaction between surgery, inflammation and an Alzheimer-prone brain produce an acceleration of pathology that cannot be compensated for. The IL-10 results are intriguing in this regard. IL-10 is considered to be anti-inflammatory, and generally follows the pro-inflammatory response, presumably in order to dampen it. The lack of this response in the 3xTgAD model suggests a dysfunctional anti-inflammatory mechanism, perhaps explaining the continued and durable effects on tau and cognition. Figure 7 is a hypothetical model for the relationships between surgery, inflammation and long term cognitive decline. Anesthesia is included in this figure with surgery, because of the inseparable nature, and to recognize the common use of more provocative drugs, such as isoflurane. Regardless of the underlying mechanism, the results reported herein have implication for surgery in the all of our elderly patients, since we do not yet routinely test for Alzheimer disease risk factors. If these results are reproduced and translate to people, then further research in animals will be required to develop protective strategies, such as the routine perioperative use of anti-inflammatory drugs in elderly patients.

In summary, we demonstrate that the combination of surgery and anesthesia produces a persistent and possibly irreversible decrement in memory and learning in a murine model of Alzheimer disease, which is associated with initial neuroinflammation and an increase in Alzheimer neuropathology, especially tauopathy.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was funded by the National Institutes of Health NIA AG031742 (RGE) and the Austin Lamont Endowment Fund (RGE). The authors would like to especially thank Angelina Sylvestro for brain embedding and sectioning, Avner Dagan, MD for assistance with immunohistochemical staining and Myra Eckenhoff for assistance with imaging.

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Figure 1.

Time line for the post-operative Morris water maze testing. Anesthetic exposures and surgery occur at time point 0, and a few animals are euthanized 24 hours later. Cued training begins 1 week later. Actual behavioral data collection does not begin until week 2, requiring 5 days. The second cognitive testing period begins at 12 weeks post-operative and is more extensive than the first, requiring almost 3 weeks. The roto-rod motor testing was performed at 16 wk PO after the completion of the MWM testing.

Figure 2.

Reference memory testing. The escape latency (A–D) and path length (E–H) recorded during the reference memory testing for the 3xTgAD and wild type (WT) mice at two post-operative (PO) time points after surgery (Surg) or desflurane alone (Des) compared to controls (Con). No changes were found in the escape latencies for WT mice at either 2 or 13 wk PO (A, C), but significant treatment effects were found for the 3xTgAD surgical mice at both 2 wk and 13 wk PO compared to controls, with test days 2–5 showing significantly longer escape latencies with Bonferroni posttests (B, D). A non-significant trend was found at 2 weeks for the desflurane group compared to controls (Day 2, $P = 0.093$; Day 4, $P = 0.057$). The mean path length during the reference memory testing also showed an overall

significant surgical effect at both 2 and 13 wk PO for the 3xTgAD surgical mice (F, H), with 2 test days showing significantly different path lengths. Animals numbers for panels A and E, WT: Con n=14, Des n=14, Surg n=16; panels B and F, 3xTgAD: Con n=40, Des n=14, Surg n=16; panels C and G, WT: Con n=25, Des n=12, Surg n=14; panels D and H, 3xTgAD: Con n=34, Des n=12, Surg n=12. Key in panel A is the same for all panels, black (control), blue (desflurane), red (surgery). For all graphs, data expressed as means $\pm$ SEM. Significance determined for both analyses by repeated measures 2-way ANOVA with Bonferroni post-hoc testing, *P<0.05, **P<0.01, ***P<0.001.

Figure 3.

Probe and spatial working memory tests. (A, B) When probe test results are compared by genotype, the mean of the ratios of the percent time spent in the target quadrant to the opposite quadrant, a decline in all of the 3xTgAD mice is apparent at 2 wks. At 13 wk PO, a significant decline was observed in the 3XTgAD surgical mice as compared to their controls (B). (C) Spatial working memory conducted beginning at 14 weeks PO showed that the surgery 3xTgAD mice performed significantly less well than their controls. (D, E) Swim speeds recorded during the 60 s probe test at 2 and 13 wk PO showed the wild type anesthetic and surgical mice and the 3xtgAD surgical mice had decreased swim speeds compared to controls though the Roto-rod data (F) indicates that all mice have the same motor ability. Animals numbers for panels A & D, WT: Con n=14, Des n=14, Surg n=16; 3xTgAD: Con n=40, Des n=14, Surg n=16; Panels B, C, E & F, WT: Con n=25, Des n=12, Surg n=14; 3xTgAD: Con n=34, Des n=12, Surg n=12. Key in panel A is the same for all panels. For all graphs, data expressed as means $\pm$ SEM. Statistical significance determined by 2-way ANOVA with Bonferroni post tests; *P<0.05, **P<0.01, ***P<0.001.

Figure 4.

Immunohistochemistry of Alzheimer markers in the 3xTgAD mouse subiculum at 16 wk PO. Panels A–C are representative sections stained for amyloid β (6E10 antibody) from the control, desflurane and surgery groups respectively, with the quantitation shown in panel G. Controls (n= 13, white bar), desflurane (n= 13, grey bar) and surgery (n= 12, black bar). Panels D–F are representative sections of immunohistochemical localization of pTau (AT8 antibody) for control, desflurane and surgery respectively, with the quantitation shown in panel H. Controls (n= 13, white bar), desflurane (n= 14, grey bar) and surgery (n= 11, black bar), *P<0.05 using 1-way ANOVA with Bonferroni post tests. For all graphs, data expressed as means $\pm$ SEM. Micron bar = 100 μ m.

Figure 5.

Immunohistochemistry of activated microglia in WT and 3xTgAD mice. Representative sections through the hippocampal CA1 region in wild type (A–C) and 3xTgAD mice (D–F) at 24h PO stained for activated microglia (Iba-1 antibody), showing control (A,D), desflurane (B,E) and surgery (C,F) micrographs. Micron bar = 100 μ m. Quantitation for WT and 3xTgAD animals at both the 24 h and 16 wk PO time points are shown in panels G and H respectively. Controls (white bars), desflurane (grey bars) and surgery (black bars). Animal numbers at 24 h PO (G) for WT controls (n=5), desflurane (n=5), surgery (n=6) and for 3xTgAD controls (n= 4), desflurane (n=6), and surgery (n=5) and at 16 wk PO (H) WT controls (n=13), desflurane (n=10), surgery (n=13) and for 3xTgAD, controls (n=14),

desflurane (n=11) and surgery (n=10). Statistical analyses used 2-way ANOVA with Bonferroni post test, *P<0.05. For all graphs, data expressed as means $\pm$ SEM.

Figure 6.

Early (24 h PO) changes in brain cytokines/chemokines using Luminex assays. Controls (white bars), desflurane (gray bars), and surgery (black bars), n=12 for all groups. (A) Desflurane exposure increased IL-10 in both WT and 3xTgAD animals, but the addition of surgery significantly increased IL-10 further in the WT animals, and abolished the increase in 3xTgAD mice. (B) Likewise, MIG levels were found to be increased in the desflurane exposures only, and the 3xTgAD animals had a significantly larger response in general as compared to WT. (C–E) No significant treatment or genotype effects could be detected in IL-1β, TGFβ or TNFα. Statistical analyses used 2-way ANOVA with Bonferroni post test, *P<0.05.

Figure 7.

Hypothetical model for surgery induced cognitive decline. Even though desflurane alone had little durable effect, “anesthesia” is included with surgery to denote their inseparable nature. BBB: blood-brain barrier.