JPP JOURNAL OF Pharmacy and Pharmacology
ROYAL PHARMACEUTICAL SOCIETY
JPP Journal of Pharmacy And Pharmacology
Research Paper

Effects of isoflurane on learning and memory functions of wild-type and glutamate transporter type 3 knockout mice

Sunam Lee[a,b], Sang-Hon Park[a,c] and Zhiyi Zuo[a]

[a]Department of Anesthesiology, University of Virginia, Charlottesville, VA, USA, [b]Department of Anesthesiology, Korea Cancer Center Hospital, Seoul and [c]Department of Anesthesiology and Pain Management, Seoul National University Bundang Hospital, Bundang-Gu Seongnam, South Korea

Keywords
glutamate transporters; isoflurane; learning; memory; mice

Correspondence
Zhiyi Zuo, Department of Anesthesiology, University of Virginia, 1 Hospital Drive, PO Box 800710, Charlottesville, VA 22908-0710, USA.
E-mail: zz3c@virginia.edu

Received May 17, 2011
Accepted October 11, 2011

doi: 10.1111/j.2042-7158.2011.01404.x

The research work was performed in and should be attributed to the Department of Anesthesiology, University of Virginia, Charlottesville, VA 22908, USA.

Abstract

Objectives General anesthetics may contribute to the post-operative cognitive dysfunction. This study was designed to determine the effects of isoflurane on the learning and memory of healthy animals or animals with a decreased brain antioxidative capacity.

Methods Seven- to nine-week-old female CD-1 wild-type mice or glutamate transporter type 3 (EAAT3) knockout mice whose brains have a decreased glutathione level were exposed to or were not exposed to 1.3% isoflurane for 2 h. They were subjected to fear conditioning or Barnes maze tests 1 week later.

Key findings The EAAT3 knockout mice had less freezing behaviour than the wild-type mice in tone-related fear. Isoflurane did not affect the freezing behaviour of the wild-type and EAAT3 knockout mice. The time for the wild-type and EAAT3 knockout mice to identify the target hole in the training sessions and memory test with the Barnes maze was not affected by isoflurane. However, the EAAT3 knockout mice took longer to identify the target hole than the wild-type mice in these tests.

Conclusions These results suggest that EAAT3 knockout mice have significant cognitive impairment. Isoflurane may not significantly affect the cognition of wild-type and EAAT3 knockout mice in a delayed phase after isoflurane exposure.

Introduction

Postoperative cognitive dysfunction (POCD) is a recognized clinical phenomenon that presents with a decline of cognitive functions after anesthesia and surgery.[1,2] It can occur in patients after cardiac and non-cardiac surgeries.[3,4] About 30–40% patients have POCD at hospital discharge after non-cardiac surgery. The incidence is ~10% for elderly patients at 3 months after surgery.[3,4] It is not clear yet whether POCD lasts for longer than 3 months after surgery. It is also not known from clinical data whether anesthesia plays an important role in POCD. Such data may be difficult to obtain because anesthesia is often associated with surgery or invasive procedures and anesthesia alone is not used in clinical practice. Under these circumstances, it is hard to determine the contribution of anesthesia to POCD.

Many laboratory studies have been performed to determine the role of anesthesia in POCD.[5–7] However, a firm conclusion has not been drawn from these studies. Whereas cognitive performance in aged rats after isoflurane and nitrous oxide anesthesia was impaired when they were tested within 3 weeks of the anesthesia,[5,6,8] the learning and memory of aged rats were not impaired at 4 months after isoflurane anesthesia.[7] These results suggest the effects of general anesthesia on cognitive functions are not long-lasting in aged rats. Although cognitive functions in young adult rats were impaired after general anesthesia with isoflurane as the main anesthetic,[8] no impairment and even some *enhancement* of cognitive functions of young adult rats also have been reported.[5,9,10] Interestingly, isoflurane has been shown to increase the production of β amyloid peptide, a peptide that may contribute to the mechanisms of Alzheimer's disease (the most common form of dementia in the elderly[11]), in mouse brains.[12] However, existing studies have shown either no change or enhancement of cognitive functions in adult mice after isoflurane exposure,[13–15] except for one study showing that exposure to 1% isoflurane, but not to any other concentrations of isoflurane, decreased the learning but not the memory function of adult mice.[16] All of these previous studies started testing mouse cognitive functions within 1–2 days of isoflurane anesthesia.

We designed this study to determine whether isoflurane affected the cognitive functions of mice in a delayed phase after isoflurane exposure because a significant number of patients still suffer from POCD in a delayed phase after anesthesia and surgery.[3,4] We also tested the effects of isoflurane on glutamate transporter type 3 (also called excitatory amino acid transporter type 3, EAAT3) knockout mice. EAAT3 is the major neuronal EAAT.[17] In addition to transporting glutamate from extracellular space into neurons, EAAT3 also takes up cysteine, the rate-limiting substrate for the synthesis of glutathione, into cells under physiological conditions.[18,19] Glutathione is the principal intracellular antioxidant. Since uptake of cysteine via EAAT3 is a major form of provision of cysteine in neurons,[20,21] EAAT3 knockout mice have a decreased glutathione level and increased oxidative stress.[21,22] Increased oxidative stress has been associated with cognitive impairment.[23,24] It is known that many neurological diseases, such as Alzheimer's disease and Parkinson's disease, and pathological conditions, such as brain ischemia-reperfusion, have decreased intracellular antioxidant levels or increased oxidative stress.[25,26] Thus, it would be interesting to know the effects of isoflurane on cognitive functions under these pathological situations. These effects in a delayed phase are particularly interesting because many neurodegenerative diseases are chronic and further deterioration in the cognitive functions of these patients by anesthesia in a delayed phase will be very detrimental to these patients. Moreover, our study on cognitive functions in a delayed phase after isoflurane anesthesia in wild-type animals and animals with increased brain oxidative stress is novel as such information has not been reported in the literature.

The Barnes maze and fear conditioning were used to assess the cognitive functions of animals in this study. Fear conditioning is a very sensitive and non-effort-dependent test of learning and memory. The Barnes maze is designed to test spatial learning and memory. Thus, the findings of these two tests will complement each other and may identify impairment of specific learning and memory functions after isoflurane exposure.

Materials and Methods

The animal protocol was approved by the Animal Care and Use Committee of the University of Virginia (Charlottesville, VA). All animal experiments were carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publications number 80–23) revised in 1996.

Animals

The EAAT3 knockout mice were descendants of the mice established by Peghinni *et al*.[27] The exon 1 of *eaat3* gene in these mice is disrupted by a neomycin resistance cassette. These mice were backcrossed with wild-type CD-1 mice for more than ten generations to generate a strain of EAAT3 knockout mice before our study. The breeding scheme included backcrossing the EAAT3 knockout mice with wild-type CD-1 mice at least once every eight generations to prevent genetic drift as recommended by the Banbury Conference.[28] The CD-1 wild-type mice were from Charles River Laboratories (Wilmington, MA).

Isoflurane exposure

Seven- to nine-week old female CD-1 wild-type or EAAT3 knockout mice were placed in a gas-tight plexiglass chamber (~1.5 l in volume) and were gassed with 3 l/min of 1.3% isoflurane in 100% oxygen for 2 h. Accurate isoflurane (Abbott Laboratories, North Chicago, IL) concentrations were delivered to the chamber by an agent-specific vaporizer. The isoflurane concentrations in the chamber were continuously monitored by a Datex infrared analyzer (Capnomac, Helsinki, Finland). The chamber was partially submersed in a 37°C water bath to maintain its temperature between 36 and 38°C. Two hours later, mice were recovered from anesthesia in the same chamber and then placed back in their cages with *ad libitum* access to food and water. Animals in the control group were placed in the chamber and were gassed with 100% oxygen for 2 h and then placed back in their cages. To abolish the influence of repeated tests on animal behaviour, separate groups of animals were prepared for the fear conditioning and Barnes maze tests. Thus no animals were subjected to both fear conditioning and the Barnes maze.

Fear conditioning test

One week after the isoflurane or oxygen exposure, mice were subjected to the fear conditioning test using the Freeze Monitor from San Diego Instruments (San Diego, CA). Briefly, each animal was placed in a test chamber wiped with 70% alcohol and subjected to three tone-foot shock pairings (tone: 2000 Hz, 75 db, 30 s; foot shock: 0.3 mA, 2 s) with an intertrial interval of 1 min in a relatively dark room. The animal was removed from this test chamber 30 s after the conditioning training. The animal was placed back in the chamber 24 h later for 5 min in the absence of tone and shock. The amount of time with freezing behaviour was recorded in this 5 min. The animal was placed 2 h later in a test chamber that had a different context and smell environment from the first test chamber (this second chamber was wiped with 1% acetic acid) in a relatively light room. After a 2-min acclimatization time, the auditory stimulus then was turned on for three cycles, each cycle for 30 s followed by a 1-min inter-cycle interval (4.5 min in total). The freezing behaviour in the 4.5 min period was recorded. Freezing behaviour was defined as absence of all movements except for respiration. Freezing behaviour assessed from the video was

scored by an observer who was blind to group assignment. These tests test hippocampus-dependent (context-related) and hippocampus-independent (tone-related) learning and memory functions.[29]

To test whether animals had any freezing behaviour under baseline conditions, wild type CD-1 or EAAT3 knockout mice that had never been exposed to isoflurane or tone-foot shock conditioning stimuli were placed in the test chamber for 5 min. Their freezing behaviours during this period were recorded.

Barnes maze test

One week after isoflurane or oxygen exposure, animals were subjected to the Barnes maze. Animals were placed in the middle of a circular platform with 20 equally spaced holes (SD Instruments, San Diego, CA). One of the holes was connected to a dark chamber that was called the target box. Animals were encouraged to find this box by aversive noise (85 dB) and bright light (200 W) shed on the platform. The animals went through a spatial acquisition phase, which took 4 days with 3 min per trial, four trials per day and 15 min between each trial. Animals then went through the reference memory phase to test short-term retention on day 5 and long-term retention on day 12. The locations of all extra-maze objects in the test room and the target hole were not changed during the training sessions, or the short- and long-term memory tests. No test was performed during the period from day 5 to day 12. The latency to find the target box during each trial was recorded with the assistance of the ANY-Maze video tracking system (SD Instruments).

Statistical analysis

Results are presented as means ± SD ($n \geq 4$). The results from the training sessions of the Barnes maze test were analyzed by two-way (CD-1 wild-type mice vs EAAT3 knockout mice, isoflurane vs no-isoflurane exposure) repeated measures analysis of variance followed by the Tukey test. All other results were tested by two-way analysis of variance followed by the Tukey test. $P \leq 0.05$ was accepted as significant. All statistical analyses were performed with the SigmaStat (Systat Software, Inc., Point Richmond, CA).

Results

None of the CD-1 wild-type and EAAT3 knockout mice ($n = 10$) presented freezing behaviour in the test chamber at baseline (before the exposure to isoflurane and fear-conditioning stimulation). There was a significant effect of mouse type (wild-type vs EAAT3 knockout) on the tone-related freezing behaviour ($P = 0.048$). The effect of mouse type on context-related freezing behaviour trended toward significance ($P = 0.183$) (Figure 1). These results suggest that

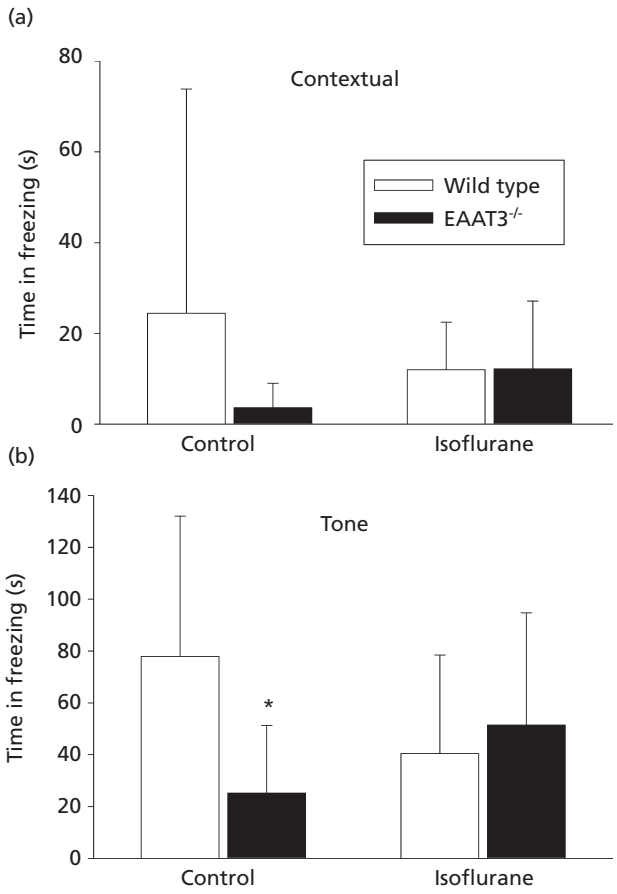

Figure 1 Performance on fear conditioning test. Seven- to nine-week-old female mice were exposed to or were not exposed to 1.3% isoflurane for 2 h. They were subjected to the fear conditioning test 1 week later. There was a significant effect of mouse type ($P = 0.048$) on the tone-related freezing behaviours. Results are mean ± SD ($n = 12$–14). *$P < 0.05$ compared with the control CD-1 wild-type group.

EAAT3 knockout mice have learning and memory impairment. The effects of isoflurane on context- and tone-related freezing behaviour were not significant ($P = 0.804$ and 0.643, respectively), suggesting that isoflurane may not significantly affect the learning and memory functions of the wild-type and EAAT3 knockout mice if assessed by fear conditioning.

All four groups of mice took a shorter time to identify the target hole with the increasing training sessions in the Barnes maze tests (Figure 2, $P < 0.001$). The effects of mouse types (wild-type vs EAAT3 knockout) on the performance in these training sessions were significant ($P < 0.001$). However, the effects of isoflurane were not significant ($P = 0.688$). The EAAT3 knockout mice that were exposed or were not exposed to isoflurane also took longer than their corresponding CD-1 wild-type mice to identify the target hole during the short-term retention test. Although the EAAT3 knockout mice that were not exposed to isoflurane needed a longer time than the

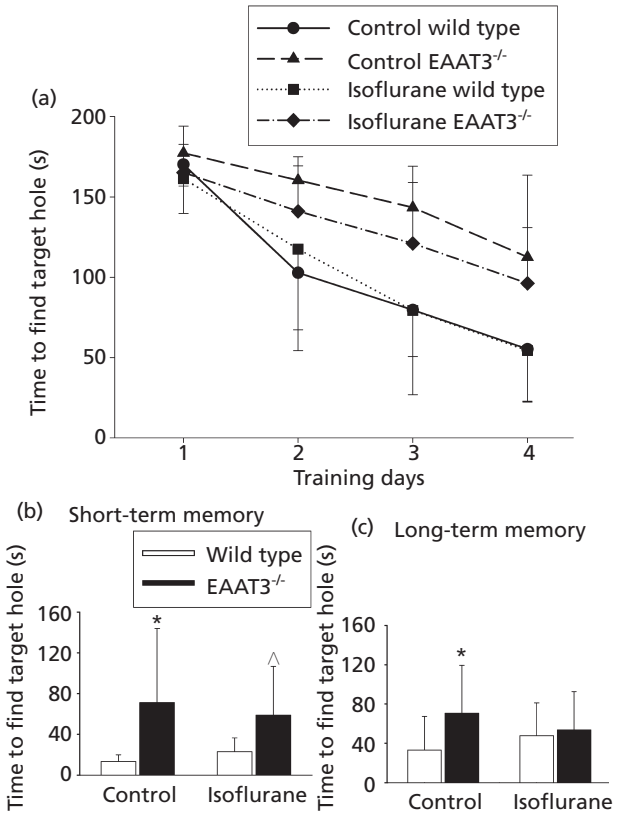

Figure 2 Performance on Barnes maze. Seven- to nine-week-old female mice were exposed to or were not exposed to 1.3% isoflurane for 2 h. They were subjected to the Barnes maze 1 week later. Results are mean ± SD (n = 4–6). In panel A, there was a significant effect of mouse type ($P < 0.001$) and training trial ($P < 0.001$) but not of the use of isoflurane ($P = 0.688$) on performance. In panel B and C, *$P < 0.05$ compared with the control CD-1 wild-type group; ^$P < 0.05$ compared with the CD-1 wild-type mice that were exposed to isoflurane.

control CD-1 wild-type mice to find the target hole in the long-term memory test, this difference disappeared when comparing the isoflurane anesthetized CD-1 wild-type mice with isoflurane anesthetized EAAT3 knockout mice (Figure 2).

Discussion

Since there is no extracellular enzyme to metabolize glutamate, glutamate uptake via EAATs is considered to be the main mechanism to prevent the accumulation of glutamate in the extracellular space under physiological conditions.[17] Five EAATs have been identified. EAAT1 and EAAT2 are expressed in glia and distributed in various brain regions. EAAT3 and EAAT4 are neuronal EAATs. EAAT3 is identified in many brain regions; EAAT4 is mainly expressed in the cerebellum. EAAT5 is expressed in the retina.[17] EAAT1 and EAAT2 are mainly expressed in the glial membrane around the synapses and are considered as the major EAATs regulating extracellular glutamate concentrations.[30,31] However, EAAT3 is expressed throughout the neurons[32] and various novel functions have been identified for it. One such function is to take up cysteine for the synthesis of glutathione in neurons. Lack of this function in EAAT3 knockout mice has been hypothesized to be the etiology for early-onset brain aging, including brain atrophy and impairment of learning and memory identified at an age of 11 months.[21]

Our results from the Barnes maze and fear conditioning test have consistently shown the impairment of learning and memory in about 2-month-old EAAT3 knockout mice. Significant brain structure changes and impairment in learning and memory were not seen in mice at this age in a previous study using the Morris water maze.[21] The identification of learning and memory impairment of these mice at young ages in our study may be due to the use of very sensitive and less stressful learning and memory paradigms. Although there may not be significant brain anatomic changes, we and others have shown that 2–3-month-old EAAT3 knockout mice have decreased antioxidative capacity.[21,22,33] In addition, EAAT3 redistribution to the plasma membrane has been found to be associated with the development of fear conditioning,[34] suggesting that EAAT3 may participate in the learning and memory process. Our results support this possibility but cannot exclude the contribution of indirect effects, such as decreased antioxidative capacity leading to changes in proteins involved in learning and memory, and the impaired learning and memory observed in the EAAT3 knockout mice.

One focus of this study is to determine the effects of isoflurane on learning and memory. Isoflurane at clinically relevant concentrations has been shown to have no significant effects or to provide an improvement in the learning and memory functions in mice.[13,15] Isoflurane also attenuates hypoxia-induced impairment of learning and memory in mice.[14] However, a recent study showed that application of 1% isoflurane (but not 1.5 or 2% isoflurane) for 1 h impaired the learning but not the memory of young adult mice.[16] These previous studies started to assess the mouse learning and memory functions within 1–2 days after isoflurane exposure. Our assessments started 1 week after the isoflurane exposure to determine the isoflurane effects in a delayed phase. We exposed our animals to 1.3% isoflurane, the minimum alveolar concentration determined in our previous study for these two types of animals.[35] Our results showed that the learning and memory in a delayed phase after the isoflurane anesthesia were not affected in CD-1 wild-type mice.

Although the possible adverse effects of volatile anesthetics on the brain are concerning in healthy individuals, the safety of using these anesthetics in individuals with various neuropathological changes is also a significant issue. Decreased antioxidative capacity and/or increased oxidative stress contribute significantly to the development of many

neurodegenerative diseases.[25,26] EAAT3 knockout mice have been proposed as a useful animal model for Parkinson's disease.[36] Our results suggest that isoflurane may not have a significant effect on the learning and memory of EAAT3 knockout mice.

Oxidative stress has been proposed to contribute to brain aging and the cognitive impairment associated with brain aging.[23,24,37] Consistent with the isoflurane having no effect on the cognitive functions of wild-type and EAAT3 knockout mice, isoflurane does not affect the production of reactive oxygen species in rat pheochromacytoma cell cultures.[38] Although isoflurane pretreatment-induced protection in rabbit spinal cord may be due to release of free radicals, this suggestion was based on the ability of a free radical scavenger to inhibit the protection; direct measurement of free radical production was not performed in that study.[39] On the other hand, our previous study shows that isoflurane may preserve cellular protein functions under oxidative stress.[40]

Our study has potential limitations. First, we did not closely monitor the physiological parameters of our mice except for maintaining their temperature when they were exposed to isoflurane. However, our previous studies showed that 1.5% isoflurane did not cause significant hypoxia in these mice.[22,41] Significant changes in physiological parameters may not have happened in our animals because our results did not show the detrimental effects of isoflurane on the learning and memory of these animals. Second, we used one clinically relevant isoflurane concentration in the study. The effect on learning and memory of isoflurane at other concentrations or in combination with other adjuvant agents used in general anesthesia is not known.

Conclusions

Our results show that EAAT3 knockout mice had significant impairment of learning and memory. Isoflurane did not have a significant effect on learning and memory in the CD-1 wild-type EAAT3 knockout mice.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

Funding

This study was supported by a grant (GM065211 to Z Zuo) from the National Institutes of Health, Bethesda, Maryland, a grant from the International Anesthesia Research Society (2007 Frontiers in Anesthesia Research Award to Z Zuo), Cleveland, Ohio, by a grant-in-aid from the American Heart Association Mid-Atlantic Affiliate (10GRNT3900019 to Z Zuo), Baltimore, Maryland and the Robert M. Epstein Professorship endowment, University of Virginia.

References

1. Moller JT *et al*. Long-term postoperative cognitive dysfunction in the elderly. International study of postoperative cognitive dysfunction. *Lancet* 1998; 351: 857–861.
2. Baranov D *et al*. Consensus statement: First International Workshop on Anesthetics and Alzheimer's disease. *Anesth Analg* 2009; 108: 1627–1630.
3. Newman MF *et al*. Longitudinal assessment of neurocognitive function after coronary-artery bypass surgery. *N Engl J Med* 2001; 344: 395–402.
4. Monk TG *et al*. Predictors of cognitive dysfunction after major noncardiac surgery. *Anesthesiology* 2008; 108: 18–30.
5. Culley DJ *et al*. The memory effects of general anesthesia persist for weeks in young and aged rats. *Anesth Analg* 2003; 96: 1004–1009.
6. Culley DJ *et al*. Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-nitrous oxide anesthesia in aged rats. *Anesth Analg* 2004; 99: 1393–1397.
7. Stratmann G *et al*. Isoflurane does not affect brain cell death, hippocampal neurogenesis, or long-term neurocognitive outcome in aged rats. *Anesthesiology* 2010; 112: 305–315.
8. Culley DJ *et al*. Long-term impairment of acquisition of a spatial memory task following isoflurane-nitrous oxide anesthesia in rats. *Anesthesiology* 2004; 100: 309–314.
9. Crosby C *et al*. Spatial memory performance 2 weeks after general anesthesia in adult rats. *Anesth Analg* 2005; 101: 1389–1392.
10. Stratmann G *et al*. Isoflurane differentially affects neurogenesis and long-term neurocognitive function in 60-day-old and 7-day-old rats. *Anesthesiology* 2009; 110: 834–848.
11. Selkoe DJ. Alzheimer's disease: genes, proteins, and therapy. *Physiol Rev* 2001; 81: 741–766.
12. Xie Z *et al*. The common inhalation anesthetic isoflurane induces caspase activation and increases amyloid beta-protein level in vivo. *Ann Neurol* 2008; 64: 618–627.
13. Butterfield NN *et al*. The effect of repeated isoflurane anesthesia on spatial and psychomotor performance in young and aged mice. *Anesth Analg* 2004; 98: 1305–1311, table of contents.
14. Bekker A *et al*. Isoflurane preserves spatial working memory in adult mice after moderate hypoxia. *Anesth Analg* 2006; 102: 1134–1138.
15. Rammes G *et al*. Isoflurane anaesthesia reversibly improves cognitive function and long-term potentiation (LTP) via an up-regulation in NMDA receptor 2B

subunit expression. *Neuropharmacology* 2009; 56: 626–636.
16. Valentim AM *et al*. Lower isoflurane concentration affects spatial learning and neurodegeneration in adult mice compared with higher concentrations. *Anesthesiology* 2010; 113: 1099–1108.
17. Danbolt NC. Glutamate uptake. *Prog Neurobiol* 2001; 65: 1–105.
18. Zerangue N, Kavanaugh MP. Interaction of L-cysteine with a human excitatory amino acid transporter. *J Physiol* 1996; 493 (Pt 2): 419–423.
19. Dringen R *et al*. Synthesis of the antioxidant glutathione in neurons: supply by astrocytes of CysGly as precursor for neuronal glutathione. *J Neurosci* 1999; 19: 562–569.
20. Dringen R. Metabolism and functions of glutathione in brain. *Prog Neurobiol* 2000; 62: 649–671.
21. Aoyama K *et al*. Neuronal glutathione deficiency and age-dependent neurodegeneration in the EAAC1 deficient mouse. *Nat Neurosci* 2006; 9: 119–126.
22. Li L, Zuo Z. Glutamate transporter type 3 knockout reduces brain tolerance to focal brain ischemia in mice. *J Cereb Blood Flow Metab* 2010; 31: 1283–1292.
23. Head E *et al*. Oxidative damage increases with age in a canine model of human brain aging. *J Neurochem* 2002; 82: 375–381.
24. Skoumalova A *et al*. The role of free radicals in canine counterpart of senile dementia of the Alzheimer type. *Exp Gerontol* 2003; 38: 711–719.
25. Lipton P. Ischemic cell death in brain neurons. *Physiol Rev* 1999; 79: 1431–1568.
26. Mates JM *et al*. Antioxidant enzymes and human diseases. *Clin Biochem* 1999; 32: 595–603.
27. Peghini P *et al*. Glutamate transporter EAAC-1-deficient mice develop dicarboxylic aminoaciduria and behavioral abnormalities but no neurodegeneration. *EMBO J* 1997; 16: 3822–3832.
28. Silva AJ *et al*. Mutant mice and neuroscience: recommendations concerning genetic background. Banbury Conference on Genetic Background in Mice. *Neuron* 1997; 19: 755–759.
29. Kim JJ, Fanselow MS. Modality-specific retrograde amnesia of fear. *Science* 1992; 256: 675–677.
30. Rothstein JD *et al*. Localization of neuronal and glial glutamate transporters. *Neuron* 1994; 13: 713–725.
31. Rothstein JD *et al*. Knockout of glutamate transporters reveals a major role for astroglial transport in excitotoxicity and clearance of glutamate. *Neuron* 1996; 16: 675–686.
32. Coco S *et al*. Non-synaptic localization of the glutamate transporter EAAC1 in cultured hippocampal neurons. *Eur J Neurosci* 1997; 9: 1902–1910.
33. Won SJ *et al*. EAAC1 gene deletion alters zinc homeostasis and exacerbates neuronal injury after transient cerebral ischemia. *J Neurosci* 2010; 30: 15409–15418.
34. Levenson J *et al*. Long-term potentiation and contextual fear conditioning increase neuronal glutamate uptake. *Nat Neurosci* 2002; 5: 155–161.
35. Lee SN *et al*. Glutamate transporter type 3 knockout mice have a decreased isoflurane requirement to induce loss of righting reflex. *Neuroscience* 2010; 171: 788–793.
36. Berman AE *et al*. N-acetylcysteine prevents loss of dopaminergic neurons in the EAAC1(-/-) mouse. *Ann Neurol* 2011; 69: 509–520.
37. Head E *et al*. Oxidative stress, aging, and central nervous system disease in the canine model of human brain aging. *Vet Clin North Am Small Anim Pract* 2008; 38: 167–178.
38. Liang G *et al*. A presenilin-1 mutation renders neurons vulnerable to isoflurane toxicity. *Anesth Analg* 2008; 106: 492–500.
39. Sang H *et al*. Isoflurane produces delayed preconditioning against spinal cord ischemic injury via release of free radicals in rabbits. *Anesthesiology* 2006; 105: 953–960.
40. Lee SA *et al*. Volatile anesthetics attenuate oxidative stress-reduced activity of glutamate transporter type 3. *Anesth Analg* 2009; 109: 1506–1510.
41. Kim J *et al*. Delayed treatment with isoflurane attenuates lipopolysaccharide and interferon γ-induced activation and injury of mouse microglial cells. *Anesthesiology* 2009; 111: 566–573.

