Published in final edited form as:

Anesth Analg. 2015 November ; 121(5): 1117–1118. doi:10.1213/ANE.0000000000000937.

Inhaled Anesthesia, Apoptosis, and the Developing Retina: A Window into the Brain?

Steven Roth, MD

The University of Illinois, Chicago, Illinois

In this month's issue of *Anesthesia & Analgesia*, Cheng et al¹ describe how isoflurane produces apoptosis in the retina in 7-day-old mouse pups. They found that exposure to 2% isoflurane for 1 h resulted in apoptosis in cells in the inner nuclear layer of the retina, which by double-label immunostaining, appeared to be amacrine and bipolar cells. Increasingly, anesthesiologists have become concerned about the risks of exposing the immature brain to anesthetic agents. Little data are available to guide clinical practice in this regard, although animal studies have suggested that even brief exposure may not be benign;^{2,3} accordingly, a means to more easily monitor for adverse effects of anesthetic exposure on neuronal cells would be a significant advance in the field. There are two main implications of the study by Cheng et al: first, that isoflurane promotes apoptosis in the immature retina, perhaps in a cell-specific manner; and second, that the retina may serve as an indirect means of visualizing and understanding potentially adverse effects of inhaled anesthesia on neurons in the brain.

In recent years, it has become apparent that changes in the retina provide a “window” into the brain for a number of serious, common degenerative diseases.⁴ These findings follow naturally, as the retina is formed embryologically from the central nervous system, with cells and axonal structures resembling those in the brain. The retina contains layers of specialized cells from the photoreceptors to the retinal ganglion cells (RGCs) that process light. Axons from the RGCs extend to the lateral geniculate nucleus and the superior colliculus, and from there to the visual cortex, where higher visual processing results in our ability to see the world.⁵

Advances in imaging have enabled detailed visualization of retinal cell layers, axons, and retinal vasculature in humans. Examples of such techniques include ocular coherence tomography, enhanced depth imaging spectral domain ocular coherence tomography, fundus photography, and confocal scanning laser ophthalmoscopy.^{6–8} Adaptive optics is another

Corresponding Author: Steven Roth, MD, Professor of Anesthesiology and Ophthalmology, The University of Illinois, Professor Emeritus of Anesthesia and Critical Care, The University of Chicago, Chicago, Illinois, Voice: 312-996-0052, rothgas@uic.edu; Address reprint requests to Dr. Roth, Dept. of Anesthesiology, University of Illinois, 1740 West Taylor Street, Room 3200, Chicago, IL 60612.

The author declares no conflict of interests.

Disclosures: Name: Steven Roth, MD

Contribution: This author wrote the manuscript.

Attestation: Steven Roth approved the final manuscript.

This manuscript was handled by: Gregory Crosby, MD

technique that compensates for aberrations in the optical path, used successfully to image photoreceptors in monkeys and humans.⁹

While direct visualization of RGCs has been utilized for decades in experimental models, the technology has not yet been translated for humans. Injection of tracers into the vitreous that hone in on and visualize RGCs is now feasible, and undergoing clinical trials. Importantly, one of the techniques, known as DARC (Detection of Apoptotic Retinal Cells) visualizes apoptotic RGCs.¹⁰ In theory, such techniques may allow clinicians to follow the progression of retinal diseases including glaucoma, diabetic retinopathy, macular degeneration, and photoreceptor degeneration, among others,¹¹ and as a guide to treatment efficacy.

The significance of the study by Cheng et al. is that changes in the retina may be reflective of what is happening in the brain. While a weakness of the study is that the authors did not directly compare isoflurane-induced changes in retinal cells to those in the brain, it has been recognized for centuries that the eye may serve as a window into the brain. Intense interest has focused on the retinal manifestations of brain degenerative diseases including Alzheimer and Parkinson diseases (AD and PD), multiple sclerosis, and vascular diseases including stroke. In particular, retinal microvascular abnormalities can predict stroke.¹² Both AD and PD cause retinal cell loss, and thinning of the retinal nerve fiber layer.^{8,13} Tau and amyloid beta proteins increase in retinae of AD subjects.¹⁴ In age-related macular degeneration, a retinal disease, molecules present in drusen are also found in AD plaques.¹⁵ RGC loss precedes axonal atrophy and disturbed axonal transport in glaucoma, a similar pattern to that of AD and PD.¹⁶ Overall, these vascular and neurodegenerative disease studies support the translational potential for “indexing” cerebral pathology by examinations of the neural retina.

Apart from investigations of blood flow, few reports have addressed the potentially adverse effects of inhaled anesthetics on the retina.^{17,18} In the present study, Cheng et al. exposed 7-day-old mouse pups to 2% isoflurane or air for 1 h. Five h after exposure, increased TUNEL (Terminal deoxynucleotidyl transferase-mediated UTP nick-end labeling) staining, as well as caspase-3 activation (both indicators of apoptotic cells) were observed in the retina's inner nuclear layer. Caspases-8 and 9 were increased in the RGC layer. The authors found that caspase-3 co-localized with immunostaining markers for bipolar cells and amacrine cells, both of which are found in the inner nuclear layer. Finally, isoflurane increased the percentage of cells in the inner nuclear layer that bound Annexin V, a phospholipid binding protein used to detect apoptosis. Although conducted in mice, these findings may carry significant implications for our pediatric patients.

Hopefully the study by Cheng et al. will prompt further examination of the impact of anesthetic agents on retinal structure/function in developing animals. Several questions come to mind: In particular, what is the impact of longer periods of exposure? What are the effects of IV anesthetic agents? Does retinal cell death temporally reflect that occurring in the brain, and is such an index reliable? More specific to the findings reported in the present study: Why are TUNEL and Annexin binding after isoflurane exposure primarily found in the inner nuclear layer and not in the RGC layer? Of all retinal cells, the RGCs and their axonal

projections most closely resemble those in the brain, and in fact, RGC axons project into the brain. That TUNEL and caspase-3 and caspase-8 expression did not exhibit temporal correlations in the present study, nor did Annexin binding with TUNEL, suggests that some immunolabels and/or methodologies may provide more accurate insights into retinal cell apoptosis than others.

A significant limitation of this study is that anesthetic agent-induced apoptosis has yet to be correlated with behavioral or cognitive impairment.¹⁹ Unfortunately, the authors did not provide any behavioral or neurophysiological data (e.g., electroretinography)²⁰ to support their findings. The relevance of this model to humans is not clear, because the postnatal day 7 age in mice may be more analogous to a late gestation time point in humans.²¹

These limitations aside, the authors have certainly opened up a new area worthy of investigation, in which the possibility exists to examine the impact of anesthetic agents on brain development using the retina as a metric. If indeed retinal changes from anesthetic agents in the immature central nervous system mirror those in the brain, it may one day be possible to study such changes in real time using retinal imaging, and thereby provide early warning of potential deleterious effects in individual patients.

Acknowledgments

Funding: Supported in part by National Institutes of Health (Bethesda, MD) grant EY10343 to Dr. Roth.

References

1. Cheng Y, He L, Prasad V, Wang S, Levy RJ. Anesthesia-induced neuronal apoptosis in the developing retina: A window of opportunity. *Anesth Analg*. 2015 IN THIS ISSUE.
2. Amrock LG, Starner ML, Murphy KL, Baxter MG. Long-term effects of single or multiple neonatal sevoflurane exposures on rat hippocampal ultrastructure. *Anesthesiology*. 2015; 122:87–95. [PubMed: 25289484]
3. Jevtovic-Todorovic V, Absalom AR, Blomgren K, Brambrink A, Crosby G, Culley DJ, Fiskum G, Giffard RG, Herold KF, Loepke AW, Ma D, Orser BA, Planel E, Slikker W Jr, Soriano SG, Stratmann G, Vutskits L, Xie Z, Hemmings HC Jr. Anaesthetic neurotoxicity and neuroplasticity: an expert group report and statement based on the BJA Salzburg Seminar. *Br J Anaesth*. 2013; 111:143–51. [PubMed: 23722106]
4. London A, Benhar I, Schwartz M. The retina as a window to the brain—from eye research to CNS disorders. *Nat Rev Neurol*. 2013; 9:44–53. [PubMed: 23165340]
5. Newman NJ. Neuro-ophthalmology: the afferent visual system. *Curr Opin Neurol*. 1993; 6:738–46. [PubMed: 8293145]
6. Larrosa JM, Garcia-Martin E, Bambo MP, Pinilla J, Polo V, Otin S, Satue M, Herrero R, Pablo LE. Potential new diagnostic tool for Alzheimer's disease using a linear discriminant function for Fourier domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2014; 55:3043–51. [PubMed: 24736054]
7. Gharbiya M, Trebbastoni A, Parisi F, Manganiello S, Cruciani F, D'Antonio F, De Vico U, Imbriano L, Campanelli A, De Lena C. Choroidal thinning as a new finding in Alzheimer's disease: evidence from enhanced depth imaging spectral domain optical coherence tomography. *J Alzheimer's Dis*. 2014; 40:907–17. [PubMed: 24577467]
8. Marziani E, Pomati S, Ramolfo P, Cigada M, Giani A, Mariani C, Staurengi G. Evaluation of retinal nerve fiber layer and ganglion cell layer thickness in Alzheimer's disease using spectral-domain optical coherence tomography. *Invest Ophthalmol Vis Sci*. 2013; 54:5953–8. [PubMed: 23920375]

9. Carroll J, Kay DB, Scoles D, Dubra A, Lombardo M. Adaptive optics retinal imaging--clinical opportunities and challenges. *Curr Eye Res.* 2013; 38:709–21. [PubMed: 23621343]
10. Galvao J, Davis BM, Cordeiro MF. In vivo imaging of retinal ganglion cell apoptosis. *Curr Opin Pharmacol.* 2013; 13:123–7. [PubMed: 22995681]
11. Barnett EM, Zhang X, Maxwell D, Chang Q, Piwnica-Worms D. Single-cell imaging of retinal ganglion cell apoptosis with a cell-penetrating, activatable peptide probe in an in vivo glaucoma model. *Proc Natl Acad Sci USA.* 2009; 106:9391–6. [PubMed: 19458250]
12. Wong TY, Klein R, Couper DJ, Cooper LS, Shahar E, Hubbard LD, Wofford MR, Sharrett AR. Retinal microvascular abnormalities and incident stroke: the Atherosclerosis Risk in Communities Study. *Lancet.* 2001; 358:1134–40. [PubMed: 11597667]
13. Moreno-Ramos T, Benito-Leon J, Villarejo A, Bermejo-Pareja F. Retinal nerve fiber layer thinning in dementia associated with Parkinson's disease, dementia with Lewy bodies, and Alzheimer's disease. *J Alzheimer's Dis.* 2013; 34:659–64. [PubMed: 23271313]
14. Ho WL, Leung Y, Tsang AW, So KF, Chiu K, Chang RC. Review: tauopathy in the retina and optic nerve: does it shadow pathological changes in the brain? *Mol Vis.* 2012; 18:2700–10. [PubMed: 23170062]
15. Yoshida T, Ohno-Matsui K, Ichinose S, Sato T, Iwata N, Saido TC, Hisatomi T, Mochizuki M, Morita I. The potential role of amyloid beta in the pathogenesis of age-related macular degeneration. *J Clin Invest.* 2005; 115:2793–800. [PubMed: 16167083]
16. Buckingham BP, Inman DM, Lambert W, Oglesby E, Calkins DJ, Steele MR, Vetter ML, Marsh-Armstrong N, Horner PJ. Progressive ganglion cell degeneration precedes neuronal loss in a mouse model of glaucoma. *J Neurosci.* 2008; 28:2735–44. [PubMed: 18337403]
17. Roth S, Pietrzyk Z, Crittenden AP. The effects of enflurane on ocular blood flow. *J Ocul Pharmacol.* 1993; 9:251–6. [PubMed: 8228532]
18. Roth S. The effects of halothane on retinal and choroidal blood flow in cats. *Anesthesiology.* 1992; 76:455–60. [PubMed: 1539858]
19. Liang G, Ward C, Peng J, Zhao Y, Huang B, Wei H. Isoflurane causes greater neurodegeneration than an equivalent exposure of sevoflurane in the developing brain of neonatal mice. *Anesthesiology.* 2010; 112:1325–34. [PubMed: 20460994]
20. Dreixler JC, Poston JN, Balyasnikova I, Shaikh AR, Tupper KY, Conway S, Boddapati V, Marcet MM, Lesniak MS, Roth S. Delayed administration of bone marrow mesenchymal stem cell conditioned medium significantly improves outcome after retinal ischemia in rats. *Invest Ophthalmol Vis Sci.* 2014; 55:3785–96. [PubMed: 24699381]
21. Dobbing J, Sands J. Quantitative growth and development of human brain. *Arch Dis Child.* 1973; 48:757–67. [PubMed: 4796010]