ANESTHESIOLOGY

Perioperative Neurocognitive Disorder

State of the Preclinical Science

Roderic G. Eckenhoff, M.D., Mervyn Maze, M.B., C.H.B., Zhongcong Xie, M.D., Ph.D., Deborah J. Culley, M.D., Sarah J. Goodlin, M.D., Zhiyi Zuo, M.D., Ph.D., Huafeng Wei, M.D., Ph.D., Robert A. Whittington, M.D., Niccolò Terrando, Ph.D., Beverley A. Orser, M.D., Ph.D., Maryellen F. Eckenhoff, Ph.D.

ANESTHESIOLOGY 2019; XXX:00–00

ABSTRACT

The purpose of this article is to provide a succinct summary of the different experimental approaches that have been used in preclinical postoperative cognitive dysfunction research, and an overview of the knowledge that has accrued. This is not intended to be a comprehensive review, but rather is intended to highlight how the many different approaches have contributed to our understanding of postoperative cognitive dysfunction, and to identify knowledge gaps to be filled by further research. The authors have organized this report by the level of experimental and systems complexity, starting with molecular and cellular approaches, then moving to intact invertebrates and vertebrate animal models. In addition, the authors' goal is to improve the quality and consistency of postoperative cognitive dysfunction and perioperative neurocognitive disorder research by promoting optimal study design, enhanced transparency, and "best practices" in experimental design and reporting to increase the likelihood of corroborating results. Thus, the authors conclude with general guidelines for designing, conducting and reporting perioperative neurocognitive disorder rodent research.

(ANESTHESIOLOGY 2019; XXX:00–00)

Patients over the age of about 65 yr are the largest consumers of procedural care.[1] Impairments in cognitive ability are the most common complications experienced in the postoperative period by these older individuals.[2,3] These impairments include postoperative delirium, occurring in the hours to days after surgery, as well as more durable deficits in executive function, memory, and other cognitive domains. The duration of cognitive impairment is variable, with most symptoms resolving in weeks to months, but in a minority the impairment continues or reemerges.[4,5] Previously, all forms of impairment were called postoperative cognitive dysfunction, but more recently, a recommended change to perioperative neurocognitive disorders has been made.[6,7] This change better aligns these disorders with the phenotypically similar neurocognitive diagnoses listed in the *Diagnostic and Statistical Manual of Mental Disorders*, version 5, such as Alzheimer disease[8–14] and Parkinson disease.[15] Clinical studies have identified age, infection, and preexisting cognitive disorders as consistent risk factors for perioperative neurocognitive disorder[6]; perioperative features, such as surgery duration, anesthetic management, and intraoperative physiology (*e.g.,* hypotension, hypoxemia) have not been rigorously implicated. In fact, other than the most acute forms of dysfunction (*e.g.,* postoperative delirium), the relationship of postoperative cognitive impairment with the surgery or anesthetic itself remains uncertain. Thus, despite consensus on the existence and character of perioperative neurocognitive disorder, whether anesthesia and surgery can be considered as etiologies, especially of the most persistent forms, has been the subject of controversy.[16]

Mechanistic interpretations of patient outcomes always suffer from the enormous complexity of patient care settings and medical interventions, as well as the diverse genetic and environmental influences that patients bring to these settings. Because the ability to dissect all these factors in humans is limited, researchers have turned to various preclinical models to reveal underlying causation and mechanisms. In this approach, ideas flowing from patient observations and mechanisms flowing from the preclinical observations can be tested and confirmed in models of appropriate complexity, with the long-range goal of optimizing perioperative brain health.

The purpose of this review is to provide a succinct summary of the different approaches used in preclinical perioperative neurocognitive disorder research and to offer an overview of the knowledge that has accrued. This report is not intended to be a comprehensive review, but rather to highlight how the different approaches have contributed to our understanding of perioperative neurocognitive disorder, and to identify knowledge gaps that need to be

Submitted for publication March 28, 2019. Accepted for publication July 25, 2019. From Anesthesiology and Critical Care, University of Pennsylvania Perelman School of Medicine, Philadelphia, Pennsylvania (R.G.E., H.W., M.F.E.); Department of Anesthesia and Perioperative Care, University of California San Francisco, San Francisco, California (M.M.); Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts (Z.X.); Department of Anesthesiology, Perioperative and Pain Medicine, Brigham and Women's Hospital, Boston, Massachusetts (D.J.C.); Harvard Medical School, Boston, Massachusetts (Z.X., D.J.C.); Department of Medicine, Oregon Health and Science University and Veterans Administration Portland Health Care System, Portland, Oregon (S.J.G.); Department of Anesthesiology, University of Virginia School of Medicine, Charlottesville, Virginia (Z.Z.); Department of Anesthesiology, Columbia University Irving Medical Center, New York, New York (R.A.W.); Department of Anesthesiology, Duke University School of Medicine, Durham, North Carolina (N.T.); and Department of Anesthesia, University of Toronto, Toronto, Canada (B.A.O.).

 Anesthesiology 2019; XXX:00–00. DOI: 10.1097/ALN.0000000000002956

<zdoi;. DOI: 10.1097/ALN.0000000000002956>

addressed by further research. Finally, our goal is to improve the quality of research in the field by promoting optimal study design, enhanced transparency and consistency, and advocacy for "best practices" in reporting to increase the likelihood of reproducing and translating results. We have organized this brief report by the level of experimental and systems complexity, starting with molecular and cellular approaches, then moving to intact invertebrates and vertebrate animal models. In the end, we provide general guidelines for designing, conducting and reporting perioperative neurocognitive disorder rodent research. These suggestions are not intended to be overly prescriptive or to stifle creativity, but rather to provide helpful guidelines that will enhance reproducibility and translatability.

In Vitro Models Used to Study Perioperative Neurocognitive Disorder

Molecular

Experimental models that examine the consequences of exposure to an anesthetic drug at the molecular level offer several key advantages. This reductionist approach allows the number of variables to be limited, and directly manipulated, and thus offers the advantage of testing mechanistic hypotheses. On the other hand, molecular studies have the disadvantage of being limited in their ability to translate to behavioral correlates. Generally, the approach allows for high-throughput studies, where several factors such as key target receptors and components in cell signaling pathways can be explored.Variability between experiments can include biologic variation but generally reflects only technical variation. Examples here were the demonstration that some general anesthetics accelerate the aggregation of the Alzheimer disease–associated amyloid β[8,17] protein, through a defined biophysical mechanism.[18] Given the phenotypic similarity between Alzheimer disease and some forms of perioperative neurocognitive disorder, these studies set the stage for discussion below on cell and animal studies (see Cell Culture Models and Animal Models of Postoperative Cognitive Dysfunction and Perioperative Neurocognitive Disorder sections), which has focused on amyloidopathy as a possible cause of cognitive impairment. The available molecular data at this level are relatively sparse. For example, we do not know if or how anesthetics interact with isolated tau, tau/tubulin assemblies, synuclein, or TDP-43,[19,20] all of which are neurodegenerative disease–associated proteins. Also, while considerable information exists on the interaction of anesthetics with certain integrins and other components of the innate immune response,[21–24] it remains unclear if or how anesthetics interact with, for example, the many interleukins and damage-associated molecular patterns, small proteins, and their receptors that trigger or sustain an inflammatory response. It is important to note that this very reductionist approach eliminates important macromolecular interactions normally present in the cellular milieu, and cannot mimic anything resembling the complexity of surgery. However, key factors such as pH, oxygen levels, and temperature need to be considered when adopting these models.

Many membrane-associated proteins, especially ion channels, are both anesthetic targets and key participants in innate immune or cognitive responses[21] and thus have been implicated in perioperative neurocognitive disorder by association. Thus, *in situ* molecular approaches have examined complex proteins such as transmitter- or voltage-gated ion channels, when isolated in their membrane environment using techniques such as electrophysiology and high-resolution microscopy. Such ion channels may include those expressed in neurons and glia, as well as circulating immune cells. Many, but not all, intermolecular interactions such as regulatory protein–protein interations are preserved in these studies. In general, the effects of anesthetics on the activity of ion channels, such as γ-aminobutyric acid type A ($GABA_A$) receptors, *N*-methyl-D-aspartate receptors, hyperpolarization-activated cyclic nucleotide–gated channels, tandem-pore potassium channels, and transient receptor potentials, among others, have been studied[25]; however, these studies have primarily focused on identifying potential targets that mediate the desired clinical properties of anesthetics rather than potential "neurotoxic" properties. However, molecular actions do not always translate to the expected desired or undesired behavioral effects, especially when multiple such actions exist concurrently. For example, the neuronal inositol 1,4,5 trisphosphate receptor and the ryanodine receptors are calcium-release channels in the endoplasmic reticulum that are activated by most of the volatile anesthetics. The resultant elevation in intracellular calcium could contribute to the hypnotic effects of the drugs depending on placement within a neuronal circuit; however, the increase in calcium could also trigger mitochondriopathy, apoptosis, and other forms of cell death. In fact, dantrolene, a ryanodine receptor inhibitor, is being investigated as a therapy for neurodegeneration,[26,27] but has not yet been explored in intact animal models of postoperative cognitive dysfunction and perioperative neurocognitive disorder. If therapeutic, dantrolene could be deployed in humans readily since it is already in the anesthesiologist's armamentarium. In isolated cases, the effect of specific ion channels (*e.g.,* α5 subunit-containing $GABA_A$ receptor) has translated to produce something like delayed neurocognitive recovery, a diagnosis under the new perioperative neurocognitive disorder terminology (see below under "Rodents [Mice and Rats]").

Anesthetic exposures in these molecular approaches require special consideration, especially for the volatile anesthetics, as solubility is both limited and temperature-dependent. For example, if equilibrated from a gas phase (*e.g.,* bubbling), the concentrations achieved at room temperature (~22°C) could be three- to fivefold higher than those achieved at physiologic temperature (37°C). This is less of a problem if solutions are prepared by direct mixing of liquid anesthetic and buffers, although care must be taken to assure

the liquid is fully solubilized before use, and that the mixture is stored in the absence of a gas phase. For the injectable anesthetics, the solution should not include cosolvents or emulsifiers as in the case of the clinical propofol preparation, as these hydrophobic phases alter partitioning, making free drug concentrations unpredictably different than intended.

Nevertheless, like the strictly molecular approach, *in situ* molecular studies fail to mimic all the potentially contributing factors that occur during surgery, such as stress and inflammation.

Cell Culture Models

Cell culture models represent an enormous increment in complexity as compared to molecular models of single proteins. A vast array of different cell types have been studied in the quest to understand the basis of anesthetic-induced perioperative neurocognitive disorder.[10,28–40] Stable cell lines (*e.g.*, Chinese hamster ovary, human embryonic kidney, neuroglioma), isolated primary cells (*e.g.*, hippocampal neurons grown in dissociated cell cultures), stem cells (*e.g.*, neural or mesenchymal progenitor cells, and human-induced pluripotent stem cells and derived neurons), and three-dimensional cell cultures (*e.g.*, minibrain models) have been, or could be, exposed to a variety of different anesthetic drugs, at a wide range of concentrations. Cell-based approaches have the advantage of ease, speed, and being highly mechanistic, but suffer from several limitations as listed below. It is paramount for cell culture studies to verify the identity of cell lines and adhere to standards for authentication, handling, and reporting.

First, biologic variability is difficult to assess, as most such studies start with pooled cells, or immortal cell lines, in which all cells are essentially identical. At the same time, the ability to genetically transform cells is a distinct advantage as genetically altered cells can help to dissect pathways that are important to some measurable adverse process (*e.g.,* apoptosis, autophagy).

Second, anesthetic exposure conditions are uncertain, especially with the volatile compounds as the gas/liquid equilibration is slow and temperature-dependent as mentioned above (see Molecular section); media/gas partitioning is rarely measured.

Third, anesthetic concentrations that are required to induce "toxicity" in cell culture are often considerably higher than those that are administered clinically to intact animals or humans. This is a likely result of toxicity in animals being caused by physiologic disruption (hypoventilation and hypotension, among others), which is challenging to mimic in isolated cells.

Fourth, for the injectable anesthetics like propofol, it is important to limit or eliminate cosolvents and emulsifiers (*e.g.,* intralipid) that brain cells in intact animals would not be exposed to, and that complicate calculation of free drug, as mentioned above (see Molecular section).

Fifth, as noted above (Molecular section), it is challenging to mimic surgery, although stimulation with damage-associated molecular patterns, cytokines, chemokines or lipopolysaccharide has been reported in an attempt to reproduce some aspects of the inflammatory response,[35,41,42] and general cell stress can be induced through serum starvation or oxygen/glucose deprivation. Thus, while much has been learned from isolated cell studies, their ability to mimic the complex stress of surgery and anesthesia is limited, reducing translatability.

Finally, statistical approaches need to be considered carefully for cell culture studies. In a recent study of cell culture statistics methods (2011 to 2016), it was revealed that only 22% of studies used replicates correctly.[43] Researchers need to distinguish between biologic, experimental, and observational units, and realize that only the experimental unit refers to the sample size. In the case of biochemical studies using multiwell plates, wells of the same condition, on each day, are treated as subsamples and do not contribute to "n." Thus, the individual wells should be averaged within the same condition on each day and the n is the number of days the separate experiments were performed. The biologic "n" in primary culture will refer to the actual number of animals (if not pooled) from which the cell were isolated. Electrophysiologic studies often use a different standard, where each cell examined contributes to the "n" value. Most importantly, it is essential to state exactly what parameter the "n" value is referring to when reporting.

As examples, cell culture studies have shown that anesthetic drugs disrupt a number of different cytosolic signaling pathways, resulting in cell death,[44,45] mitochondriopathy,[46] and/or the release of cytokines or other signaling molecules either during or after anesthetic exposure.[35] Further, the enhancement of amyloidopathy and calcium dysregulation by anesthetics in cell models has been reported,[8,45,47,48] each or all of which may contribute to postoperative cognitive dysfunction or perioperative neurocognitive disorder endpoints in intact animals. Cell-based models also allow the study of drugs that might oppose any adverse effect of anesthetics. For example, dantrolene can block the anesthetic-induced activation of calcium release from ryanodine receptor,[49] and dexmedetomidine prevents the overexpression of α5-containing $GABA_A$ receptors on the neuronal surface.[29]

Brain Slice Models

Brain slice models maintain the integrity of at least some cell–cell communication and limited networks, which is another increment in complexity. Slice-based models also allow specific cell types located in discrete brain regions to be readily identified and tested. Anesthetic exposures have been shown to persistently disrupt functions of neuronal networks such as the long-term potentiation of synapses,[50–52] a cellular surrogate for memory and learning, which is possibly disrupted in postoperative cognitive dysfunction and perioperative neurocognitive disorder. In addition, slices can be obtained from genetically modified animals to define the role of specific proteins and signaling

pathways, and possibly offering insights into the heterogeneity of responses in humans.

Limitations of slice models are similar to the cell models in terms of anesthetic exposure, but in addition to an ability to measure function, strengths include an ability to assess biologic variability. Other limitations include reduced viability of slices from aged animals,[53] challenges in assessing neurogenesis, and the fact that cell–cell connections, especially long-range ones that might be most influenced by anesthetics, are invariably disrupted. Moreover, many cells are damaged, are deprived of their normal circulation, and can be covertly ischemic. Thus, it is difficult to know if responses to an intervention can be considered physiologic. Nevertheless, unlike the cell culture models, robust and relevant functions can be measured at least briefly; but evaluation of the effects of age, surgery and inflammation remain challenging.

Animal Models of Postoperative Cognitive Dysfunction and Perioperative Neurocognitive Disorder

Many animal models that range from worms to nonhuman primates have been used to study anesthetic neurotoxicity, but the most ubiquitous, tractable, and relevant have been mammalian models, primarily rats and mice. It is important here to make the distinction between studies at the two extremes of age. Considerable investigation of the effect of anesthetics on the developing animal brain has been published over the past decade, and this body of work is reviewed in detail elsewhere.[54] When referring to perioperative neurocognitive disorder and postoperative cognitive dysfunction, we focus on the effect of anesthesia and surgery on the aging animal brain specifically. No studies of perioperative neurocognitive disorder and postoperative cognitive dysfunction in nonhuman primates have been reported, and this model presents considerable disadvantages in terms of cost and life span, so will not be further discussed.

Caenorhabditis elegans (Nematode)

This small (1 mm in length) free-living nematode has been extensively studied for decades from a genetic standpoint. Specifically, this model has been used primarily to define the genetic determinants of general anesthetic drug sensitivity.[55] The advantages of this model include a very short life cycle, ease of husbandry, being an invertebrate (no Institutional Animal Care and Use Committee concerns), clear and consistent behavior, completely sequenced genome, transparency, and a structurally understood nervous system. It is also sensitive to all general anesthetics, although about five- to tenfold less sensitive than mammals.[56,57] Disadvantages include small size, making electrophysiology difficult, and a very primitive nervous system containing only 302 neurons. Whether worms can truly learn and remember is controversial, limiting relevant outcome measurements. Relevance and translatability are the primary concerns, although many biologic features first identified in this nematode have been subsequently validated in the mammal. Postoperative cognitive dysfunction and perioperative neurocognitive disorder studies are very limited. Both forward and reverse genetic designs have been used to study a wide variety of phenotypes, including aging. Although the worm has been used to define pathways and mechanisms for specific proteinopathies, such as Alzheimer disease,[58] it has not received much attention in the postoperative cognitive dysfunction and perioperative neurocognitive disorder domain, probably because of the concerns regarding translation.

Drosophila melanogaster (Fruit Fly)

While flies are not much larger than worms, their nervous systems are considerably more complex. The fruit fly has been studied extensively, although the studies are devoted largely to the genotype and phenotype relationship. Much work has been conducted in the neurodegeneration pathways,[59] but again, little in the postoperative cognitive dysfunction and perioperative neurocognitive disorder domain. In contrast, it has been a popular organism to understand the genetic determinants of general anesthetic sensitivity,[60,61] and more recently it is being used to understand polytrauma and sepsis.[62,63] The advantages are its easy husbandry, fully understood genetics, large numbers of readily available variants, short generation and life span, and lack of regulatory oversight. While the administration of volatile anesthetics is straightforward, the administration of injectable drugs is not. Similar to studies of worms, it is difficult to measure anesthetic concentrations *in vivo*.[64] Nevertheless, because of the previous and ongoing work in neurodegeneration, sepsis, and anesthesia, it seems that an opportunity to study postoperative cognitive dysfunction and perioperative neurocognitive disorder exists in the fly.

Danio rerio (Zebrafish)

Similar to the nematode and fruit fly, the zebrafish is extremely well understood from a genetic and developmental standpoint. Unfortunately, this versatile experimental model has received little or no attention in the postoperative cognitive dysfunction and perioperative neurocognitive disorder domain, or for that matter by the entire field of anesthesiology. An important advantage of the zebrafish is that, as a vertebrate, it is phylogenetically closer to mammals than the fruit fly or worm. However, as alluded to above in the Nematode and Fruit Fly sections, this requires that protocols involving fish be approved by Institutional Animal Care and Use Committees. Like flies and worms, the fish is well understood genetically, and many genetic variants are available. Anesthetic administration is more straightforward, as any drug that can be solubilized in pond water will be rapidly absorbed *via* diffusion through the skin or across the gills, and can be used for high-throughput screening.[65] There may be little advantage for the study of aging, or age-related processes like neurodegeneration, since their life

span is similar to the mouse, so the examination of aged fish becomes difficult. Nevertheless, models of Alzheimer disease–like neurodegeneration have been reported for both adult and larval zebrafish.[66] Also, behavioral measurements in the larvae are limited to stereotypical responses to various forms of stimulation, although learning and memory can be studied in the adult fish. To date, no postoperative cognitive dysfunction and perioperative neurocognitive disorder work has been reported using the zebrafish, but it should be useful for genetic dissection of the pathways involved. Otherwise, advantages over the mouse appear to be small.

Rodents (Mice and Rats)

The mouse has been the mainstay of postoperative cognitive dysfunction and perioperative neurocognitive disorder research to date, because of its size, cost, and ability to modify its genome. Rats have been used in some studies. Initial studies examined the effect of anesthetics on memory and learning, typically using some form of a maze task or fear-conditioning assays. Almost invariably, it was found that the state of anesthesia, produced largely by inhalational drugs, produced decrements in learning and memory that could be detected a few days or a week after the exposure.[9,67] In some cases, these decrements were associated with changes in histopathology or biomarkers consistent with neurodegeneration.[68,69] The largely wild-type (*e.g.,* C57BL/6) mice used in these studies were of different ages, and the exposures were very different, making comparisons difficult.

In attempts to make the models more representative of patient populations with postoperative cognitive dysfunction and perioperative neurocognitive disorder, researchers included other stresses or vulnerabilities. For example, recent studies have included aged rodents, typically 18 to 24 months of age. In older animals, postanesthesia behavioral decrements tend to be larger and more durable.[9,70,71] Moreover, since many patients come to surgery with preexisting cognitive impairments, and since wild-type rodents tend not to suffer from anything resembling Alzheimer disease, researchers have begun to repeat their studies with transgenic animals that include human Alzheimer disease–related genes. Most popular have been genes in the amyloid β pathway that enhance production and therefore increase brain levels of amyloid β (*e.g.,* Tg2576, APP/PS1). This strategy, when coupled with age, has revealed further decrements in learning and memory, although not necessarily representing neurodegeneration. Inhalational anesthetic exposure accelerated features of the histopathology, but not the learning and memory deficits.[67,72] Other transgenic animals that recapitulate tauopathy[73] or include both amyloidopathy and tauopathy (3xTgAD, hTau mice), in order to better recapitulate human disease, have even larger deficits.[74] In these animals, inhalational anesthetics produced no effect on behavior when young,[75] but a transient decrement in learning and memory when aged.[14] Isolated tauopathy models have also been studied, which show amplified consequences of being exposed to an anesthetic.[20,73]

In addition to the disease-pathway mechanisms, there are reports of canonical anesthetic mechanisms that produce delayed neurocognitive effects. For example, a portion of the hypnotic, amnestic, and immobilizing actions of many general anesthetics is thought to occur *via* enhancement or activation of $GABA_A$ receptor activity.[76,77] In receptors that contain the α5 subunit, anesthetics enhance expression in the neuronal membranes, a location where they become persistently active. This has been shown in animals to result in transient somnolence, amnesia, and cognitive impairment, similar to human delayed neurocognitive recovery.[78] Specific antagonists of the α5 $GABA_A$ receptor have been reported to improve animal behavior after anesthetic exposure.[29,78] It is not yet clear to what degree such a mechanism contributes to human perioperative neurocognitive disorder and postoperative cognitive dysfunction.

A very large advantage of the rodent over the other animal species mentioned above is the ability to include surgery, clearly a central part of the perioperative experience. Thus, most studies that have included surgery along with the anesthetic[14,79–82] have found a consistent increment in both the histopathology and biochemical evidence of neuroinflammation, and a greater decrement in the behavior. When age, a genetic vulnerability, a comorbidity, and surgery were all combined in the study design, the decrements in behavior became much more durable (more than 3 months).[14] Interestingly, despite the anesthetic having little detectable effect on its own, it appears that some anesthetics can modulate the result of having either a genetic vulnerability[83] or surgery.[81] The concept that best explains the rodent data to date is a modified "double-hit" model. In other words, in the presence of preexisting vulnerabilities (*e.g.,* age, genetic, and comorbidities), the large multifactorial stress of the surgery amplifies any ongoing central nervous system inflammation or injury, a process potentially modulated by other drugs like anesthetics.

Evidence suggests that neuroinflammation after surgery plays a key role in postoperative cognitive dysfunction and perioperative neurocognitive disorder.[84,85] The preexisting vulnerabilities mentioned above are thought to increase blood brain barrier permeability,[86] and allow the peripheral innate immune molecules, generated by surgical tissue damage, to enter the central nervous system to further enhance neuroinflammation and injury. Moreover, mice that lack genes to mount significant neuroinflammation did not develop postoperative cognitive dysfunction after anesthesia and surgery.[87,88] Even in the absence of surgery, stimulation of the innate immune response by lipopolysaccharide, or inducing sepsis, produces transient decrements in behavioral assays, reminiscent of delayed neurocognitive recovery, or "sickness behavior."[89,90] Blockage of either tumor necrosis factor α or interleukin 6 using antibodies effectively reduced rodent postoperative cognitive dysfunction, but also delayed wound healing.[79,82] More conventional antiinflammatory drugs (dexamethasone and

cyclooxygenase-2 inhibitors) given before, during, or after the procedure have yielded variable results,[91–95] a result that has shifted attention to innate processes that actively turn off or resolve inflammation. Initial studies of mice with the tibial fracture model show promising results with proresolution strategies, such as resolvin-D1 and maresin-1,[96,97] as well as bioelectronic approaches, such as electrical stimulation of the vagus nerve.[98]

The impact of anesthesia and surgery on patients with other forms of neurodegeneration, such as Parkinson disease or prion diseases, or on pre-existing cerebrovascular disease, have not been reported, despite these disorders being fairly common in aged surgical populations. Similarly, traumatic brain injury is thought to enhance vulnerability to Alzheimer disease and Parkinson disease. The effect of anesthesia and surgery on perioperative neurocognitive disorder and postoperative cognitive dysfunction in humans or animals with a history of even mild traumatic brain injury has not been reported. Moreover, an association between depression and educational and socioeconomic status with cognitive trajectories has been reported in human forms of neurodegeneration, another area intrinsically difficult to model in the preclinical domain, especially with rodents. The beneficial potential for some forms of nonpharmacologic approaches is suggested by evidence showing that environmental enrichment slows cognitive decline in a murine Alzheimer disease model,[99,100] as well as postoperative cognitive dysfunction in rodents.[101,102] Growing evidence also implicates the gut microbiome in several neuroinflammatory models, and its contribution in perioperative neurocognitive disorder is just beginning to be explored.[103]

Suggestions for Rodent Perioperative Neurocognitive Disorder and Postoperative Cognitive Dysfunction Research

The hundreds of rodent studies of perioperative neurocognitive disorder and postoperative cognitive dysfunction that appear every year in the literature are very heterogeneous in both design and results; translation has been limited. It is likely that at least a portion of the variability could be reduced by adherence to reporting guidelines, such as that promulgated for animal study of stroke in 2010 (*e.g.*, Animal Research: Reporting of *In Vivo* Experiments [ARRIVE]).[104] It should be noted that even 4 to 6 yr after publication of the Animal Research: Reporting of *In Vivo* Experiments guidelines, very few relevant preclinical studies published in the anesthesiology literature have adhered to or have even cited them.[105] In addition to those guidelines for reporting, we would also like to suggest that investigators consider the following guidelines when designing their studies. These guidelines touch on terminology, animal character, exposure control and monitoring, procedures, and finally, statistical considerations.

Terminology

The term postoperative cognitive dysfunction has been used in the clinical literature to refer to any postoperative cognitive dysfunction, usually in a research context, and regardless of magnitude, timing or duration. Unfortunately, the same has been true in the preclinical literature. As mentioned in the introduction, a recent set of recommendations for a new clinical nomenclature has been published in order to recognize the many inadequacies of postoperative cognitive dysfunction.[7] These recommendations were not intended for preclinical research, and cannot reliably be mapped onto, for example, performance on the fear conditioning assay, or water maze. Nevertheless, we encourage researchers to make an attempt, in the discussion or interpretation of their results, to indicate roughly where their study design fits. For example, many rodent studies have found minor but significant performance deficits out to a week or two postoperatively, with full apparent recovery thereafter. This might parenthetically be termed delayed neurocognitive recovery, even though the required "cognitive concern" cannot be voiced. In another study where the deficits appeared to be persistent even to 3 months postoperatively, this might be "neurocognitive disorder," again with the same caveat regarding the cognitive concern. Also, given that the animals could maintain their weight and other "activities of daily living" (a pretty low bar in the rodent), this would probably be closest to "mild" neurocognitive disorder. Ultimately, however, researchers simply need to be precise about what they did and why when reporting.

Animal Age, Sex, and Environment

Perioperative neurocognitive disorder and postoperative cognitive dysfunction is a syndrome of the elderly, and it is clear that the aged brain reacts differently to stresses than the young.[106,107] Thus, studies of perioperative neurocognitive disorder and postoperative cognitive dysfunction mechanisms and influences should include animals aged to at least 70 to 80% of their expected life span. It is recognized that this increases the cost and time of studies, but the tradeoff of potentially improved translation more than justifies the cost. Sex is an important biologic variable that must be considered in perioperative neurocognitive disorder and postoperative cognitive dysfunction research. Delirium and cognitive impairment are reported in both male and female patients, and thus, strong scientific justification should accompany the reporting of only a single sex. In addition to age, preexisting cognitive decline is a major risk factor for perioperative neurocognitive disorder and postoperative cognitive dysfunction, so modification to a rodent's genome or environment (drugs) that produce these cognitive impairments, while not essential, may be important to include when a researcher is establishing relevance for the human condition, as well as searching for mechanisms. Finally, a preclinical focus on persistent cognitive

impairment after surgery merits greater attention, as it is the most controversial aspect of perioperative neurocognitive disorder and postoperative cognitive dysfunction in the clinical literature.

Anesthetic Exposures

Most general anesthetics depress ventilation, body temperature, cardiac output, and blood pressure, any one of which can also have an effect on the brain in addition to any direct influences of the drug. While these physiologic perturbations are monitored and controlled in the human (or other large animal species), they are often not even monitored, let alone controlled, in typical rodent models. We suggest that, at a minimum, temperature and oxygen saturation be monitored; optimally, blood pressure and heart rate should be included. Miniaturized equipment for this purpose is now commercially available for rodents. The inhaled oxygen should be enriched to avoid hypoxemia, but probably not beyond 50% to avoid atelectasis and oxygen toxicity. While mechanical ventilation might be desirable to eliminate hypercarbia, and the accompanying respiratory acidosis, this can be prohibitively difficult in the mouse—less so in the rat. Measurement of arterial blood gases would be ideal, but the approach and blood volumes needed typically preclude survival beyond collection, and may be model-specific depending on length of anesthesia exposure or surgery duration. Most investigators who actually measure blood gases do so in sentinel animals euthanized solely for this purpose at different points in the exposure. Perhaps because it sometimes requires surgery, electroencephalographic monitoring of aged animals undergoing anesthesia has not been reported in postoperative cognitive dysfunction and perioperative neurocognitive disorder studies, but might be considered since the anesthetic sensitivity of genetically altered animals is rarely determined before using them in such studies. In addition, electroencephalographic monitoring would provide evidence of physiologic change (*e.g.,* hypotension and decreased cerebral perfusion) that could confound the results.

Mortality is not uncommon in rodent anesthetic studies, especially in aged and genetically modified animals, and in general reflects pronounced physiologic disruption that can be presumed to have existed even in the animals that survive. Thus, it is difficult to rationalize that such a study is examining the effect of the anesthetic *per se*, rather than marked physiologic trespass. Mortality due to the anesthetic is exceedingly rare in human anesthesia practice. Animal Research: Reporting of *In Vivo* Experiments guidelines should be followed to report mortality as well as any other exclusions to the experimental groups. Finally, it is not clear how long an animal exposure to anesthesia is "relevant." This should not be based on life span alone; it should be evaluated in the context of each experimental model based on previous literature, physiologic monitoring, and translational relevance.

Surgical Procedure

Reported surgical procedures in rodents have varied, and have included simple skin incision and vascular exposure, splenectomy, cecectomy and appendectomy, partial hepatectomy, and tibial fracture.[108] Other models exist but have not been explored for perioperative neurocognitive disorder, such as cardiopulmonary bypass.[109] All have merit, as postoperative cognitive dysfunction in humans has been reported after each, albeit at a different incidence and magnitude. Splenectomy may not be the best choice as it is uncommon surgery in older adults and itself modulates the innate immune response. As in human surgery, antibiotics and analgesics are used in the rodent, and while understudied, these drug classes may have a significant impact on perioperative neurocognitive disorder and postoperative cognitive dysfunction.[110]

Behavioral Assays

The earliest form of perioperative neurocognitive disorder to occur in the human after anesthesia and surgery is postoperative delirium, which, despite considerable effort, has only been partially validated in the rodent.[111,112] For example, fluctuating level of attention has been reported, but detecting disordered thinking and hallucinations in the rodent, and distinguishing them from fear, anxiety, and pain, would be necessarily arbitrary. On the contrary, there are a large number of well-validated assays of rodent learning and memory, as well as motor and coordination ability. Similarly, there are a large number of ways these assays are administered, in some cases with training beforehand, and others without a training phase at all. There are excellent reviews on the subject of animal behavior testing in aged and transgenic animals, so we will not go into detail here, but with respect to perioperative neurocognitive disorder and postoperative cognitive dysfunction, we can offer the following suggestions. Since human perioperative neurocognitive disorder and postoperative cognitive dysfunction signs and symptoms occur in multiple cognitive domains, more than a single rodent behavioral assay should be used. It is not uncommon to find no effect in one assay and significant effects in another, but the results of all assays, including those with negative findings, should be reported. Since most assays are reliant on some level of motor activity, and some surgical procedures may result in motor impairment or pain that will impact assay results and interpretation, it is useful to include independent motor ability assays, such as the rotarod. Also, transgenic and aged animals cannot be assumed to have entirely intact sensory systems (olfaction and vision, among others), on which many behavioral assays are critically dependent; baseline values and control groups are necessary. Evidence suggests that environment, including the researchers themselves,[113] influences rodent behavior, suggesting that these variables be carefully controlled. Finally, because any measure of behavior includes a degree

of subjectivity, variability will be high, indicating that a large number of animals will be required to give confidence in the results. For example, it is unlikely that group sizes of five or six animals will be sufficient to detect anything but a type I error.

Statistical Methods

Statistical methods vary greatly depending on the experimental model and design. Preclinical studies often have the advantage of low biologic variability, which reduces the numbers of cells or animals necessary to show significant effect sizes. However, this advantage is also a weakness, as a system with low biologic variability does not reflect typical human surgical populations, partially explaining the well-known translational failure.

Sample Size. Statistical methods should be rigorously addressed during the experimental design, and not after data collection. The first step is to define primary and secondary endpoints in behavioral studies, as occurs in clinical trials. Next is a power analysis, which is typically based on pilot data and performed prospectively. This allows an estimate of the effect size, which can be used to calculate the "n" required to achieve statistical significance.[114,115] Lacking pilot data, the effect size can often be estimated from the literature, or at least from what a "clinically significant" effect size might be. For example, most would consider a 10% decrease in cognitive ability after surgery to be clinically very important, but this would be considered a very small effect size in an animal, and therefore require a large "n" to reveal it significantly. Further, estimating effect sizes from the literature could be misleading as it might be merely propagating errors. We recommend that a biostatistician be integrated into the design phase of these preclinical studies in order to power the study appropriately.

Statistical Approach. The actual test will depend on the experimental design. Student's *t* test for predetermined and independent pairs of samples (*e.g.,* a primary outcome in treated and control groups) as well as ANOVA (when more than two groups are present: two treatment groups as compared to a control) are acceptable statistical methods used in preclinical studies, but only when the data are normally distributed. When not normally distributed, which is often the case, nonparametric tests must be considered to avoid misleading results. When multiple independent tests are planned, with no *a priori* focus, such as is common in enzyme-linked immunosorbent assay (ELISA) arrays, corrections for multiple testing must be used.[115] Similarly, if multiple time points or treatments are planned, ANOVA followed by a suitable *post hoc* test is required to correct for these multiple comparisons. In addition to null hypothesis testing, it is essential to consider effect sizes and their 95% CI in order to gauge translational relevance. Finally, as outlined in the Animal Research: Reporting of *In Vivo* Experiments guidelines, all data, negative and positive, as well as statistical methods should be reported, indicating any outliers and deaths that have been excluded and the reasons for exclusion. While it is understood that a negative result reporting bias exists, such reporting is vital to avoid needless repetition and improve translatability.

Rigor and Reproducibility. Funding agencies internationally are concerned about low reproducibility and translatability, which is in large part due to underpowered sample sizes, as well as experimental designs that do not include blinding, randomization, replication, positive and negative controls, and biologic differences.[116,117] Further, critical biologic variables like age, sex, and comorbidities are often not considered, and are often difficult to include in preclinical studies. Novel approaches to experimental design should be considered,[118,119] and one should work with a biostatistician from the beginning. It bears emphasizing that once published, poorly designed studies become part of the literature and difficult to distinguish from well-designed and reported ones. Ultimately this harms all researchers through loss of time and scarce research dollars.

Conclusions

The preclinical examination of perioperative neurocognitive disorder and postoperative cognitive dysfunction has revealed much in the way of mechanistic insight into cognitive impairment after anesthesia and surgery, and several compelling hypotheses regarding neuroinflammation, inflammation resolution, and adverse anesthetic effects have emerged. Barriers to progress exist, many of which lie in the area of experimental design, consistency, reporting, and terminology. Other barriers include the experimental and animal models themselves. *In vitro*, cell and slice studies suffer from an incomplete ability to model the perioperative experience, now especially important given the growing appreciation for the impact of the surgical procedure. Barriers also exist in the modeling of human vulnerabilities in animal models, and an imprecise ability to evaluate cognitive domains affected, such as executive function, attention, and disordered thinking. These experimental shortfalls have conspired to reduce translation of research results to humans.

Nevertheless, the various preclinical models will continue to be essential to address focused questions, and collectively the answers from these various models and approaches will be highly complementary. For example, what are the upstream targets that surgery and/or anesthetics activate to produce the cascades resulting in delirium and cognitive decline? Can targeting these pathways mitigate injury? What is the impact of preexisting neuronal vulnerabilities other than Alzheimer disease, such as Parkinson disease and traumatic brain injury? Very little has been reported regarding the effects of many other aspects of the perioperative period such as different sedatives, analgesic drugs, antibiotics, changes in the gut

microbiome, sleep disruption, and immobility. Also needed is a greater focus on specific pathways within the innate immune response, such as immune cell activation, adherence, and migration, or the importance of vagal traffic. Also still in its infancy is the focus on inflammatory resolution, an area that shows promise for both prevention and, potentially, treatment of perioperative neurocognitive disorder and postoperative cognitive dysfunction. Finally, improved animal assays for delirium, socialization, and problem-solving need to be adopted, as well as models of depression, social defeat, and socioeconomic status. All of these psychosocial factors deserve special attention as research has demonstrated that environmental enrichment has overcome the cognitive deficits due to a variety of stresses. These and many other knowledge gaps (detailed in table 1) cannot be easily addressed in clinical studies; much impactful preclinical work remains.

Table 1. PND Knowledge Gaps Potentially Addressable in Preclinical Studies

- Which animal model (species, strain, surgery, and anesthesia, among others) best reproduces the clinical phenotype of each form of PND (delirium, delayed neurocognitive recovery, and neurocognitive disorder)?
- Should the known risk factors for clinical PND (age, cognitive impairment, and frailty, among others) be added to the animal model (No. 1) to enhance vulnerability for PND?
- Does cerebrovascular disease contribute to PND?
- Does perioperative cardiorespiratory instability contribute to PND?
- What are the relative roles of proinflammatory *versus* proresolving responses for the development of PND?
- What are the roles of different immunocytes and their signaling pathways in the pathogenesis of PND?
- Do anesthetics differentially modulate the blood brain barrier and neuroinflammatory response to peripheral trauma?
- Which are the brain's regions of interest for peripheral surgery-induced neuroinflammation?
- Are there transcriptional, epigenomic, or proteomic responses to anesthesia and surgery that contribute to PND?
- What is the role of the microbiome in PND, and how do perioperative factors (bowel preparation, antibiotics, anesthetics, analgesics, and diet, among others) influence it?
- Is preexisting traumatic brain injury a risk factor for PND?
- How do opioids and/or pain contribute to pathogenesis of PND?
- Does depression, anxiety, or environmental deprivation modulate PND?
- Are there biomarkers that predict progression to PND that can be used to trigger interventions?
- Are there sex differences in PND vulnerability and response to interventions?
- Can the PND clinical nomenclature be mapped onto preclinical studies?

Perioperative Neurocognitive Disorder, PND.

Acknowledgments

The authors gratefully acknowledge AARP (Washington, D.C.) and the American Society of Anesthesiologists (Schaumburg, Illinois) for supporting the initial summit meeting in Washington, DC, June 20 and 21, 2018, that ultimately led to this article.

Research Support

Support was provided solely from institutional and/or departmental sources.

Competing Interests

The authors declare no competing interests.

Correspondence

Address correspondence to Dr. R. G. Eckenhoff: Department of Anesthesiology and Critical Care, University of Pennsylvania Perelman School of Medicine, 3620 Hamilton Walk, 311 John Morgan Building, Philadelphia, Pennsylvania 19104. reckenho@pennmedicine.upenn.edu.

References

1. Hall MJ, DeFrances CJ, Williams SN, Golosinskiy A, Schwartzman A: National Hospital Discharge Survey: 2007 summary. Natl Health Stat Report 2010; 29:1–20, 24
2. Inouye SK, Marcantonio ER, Kosar CM, Tommet D, Schmitt EM, Travison TG, Saczynski JS, Ngo LH, Alsop DC, Jones RN: The short-term and long-term relationship between delirium and cognitive trajectory in older surgical patients. Alzheimers Dement 2016; 12:766–75
3. Daiello LA, Racine AM, Yun Gou R, Marcantonio ER, Xie Z, Kunze LJ, Vlassakov KV, Inouye SK, Jones RN, Alsop D, Travison T, Arnold S, Cooper Z, Dickerson B, Fong T, Metzger E, Pascual-Leone A, Schmitt EM, Shafi M, Cavallari M, Dai W, Dillon ST, McElhaney J, Guttmann C, Hshieh T, Kuchel G, Libermann T, Ngo L, Press D, Saczynski J, Vasunilashorn S, O'Connor M, Kimchi E, Strauss J, Wong B, Belkin M, Ayres D, Callery M, Pomposelli F, Wright J, Schermerhorn M, Abrantes T, Albuquerque A, Bertrand S, Brown A, Callahan A, D'Aquila M, Dowal S, Fox M, Gallagher J, Anna Gersten R, Hodara A, Helfand B, Inloes J, Kettell J, Kuczmarska A, Nee J, Nemeth E, Ochsner L, Palihnich K, Parisi K, Puelle M, Rastegar S, Vella M, Xu G, Bryan M, Guess J, Enghorn D, Gross A, Gou Y, Habtemariam D, Isaza I, Kosar C, Rockett C, Tommet D, Gruen T, Ross M, Tasker K, Gee J, Kolanowski A, Pisani M, de Rooij S, Rogers S, Studenski S, Stern Y, Whittemore A, Gottlieb G, Orav J, Sperling R: Postoperative delirium and postoperative cognitive dysfunction: Overlap and divergence. Anesthesiology 2019; 131:477–91
4. Schulte PJ, Roberts RO, Knopman DS, Petersen RC, Hanson AC, Schroeder DR, Weingarten TN, Martin

DP, Warner DO, Sprung J: Association between exposure to anaesthesia and surgery and long-term cognitive trajectories in older adults: Report from the Mayo Clinic Study of Aging. Br J Anaesth 2018; 121:398–405
5. Pedersen NLE, L.I.: Hospitalization, Surgery, and Incident Dementia. Alzheimers Dement 2019; (in press)
6. The Perioperative Neurocognitive Disorders. Edited by Eckenhoff RG, Terrando N. Cambridge, England, Cambridge University Press, 2019
7. Evered L, Silbert B, Knopman DS, Scott DA, DeKosky ST, Rasmussen LS, Oh ES, Crosby G, Berger M, Eckenhoff RG; Nomenclature Consensus Working Group: Recommendations for the Nomenclature of Cognitive Change Associated with Anaesthesia and Surgery-2018. Anesthesiology 2018; 129:872–9
8. Eckenhoff RG, Johansson JS, Wei H, Carnini A, Kang B, Wei W, Pidikiti R, Keller JM, Eckenhoff MF: Inhaled anesthetic enhancement of amyloid-beta oligomerization and cytotoxicity. Anesthesiology 2004; 101:703–9
9. Culley DJ, Baxter MG, Crosby CA, Yukhananov R, Crosby G: Impaired acquisition of spatial memory 2 weeks after isoflurane and isoflurane-nitrous oxide anesthesia in aged rats. Anesth Analg 2004; 99:1393–7
10. Xie Z, Dong Y, Maeda U, Moir RD, Xia W, Culley DJ, Crosby G, Tanzi RE: The inhalation anesthetic isoflurane induces a vicious cycle of apoptosis and amyloid beta-protein accumulation. J Neurosci 2007; 27:1247–54
11. Liang G, Wang Q, Li Y, Kang B, Eckenhoff MF, Eckenhoff RG, Wei H: A presenilin-1 mutation renders neurons vulnerable to isoflurane toxicity. Anesth Analg 2008; 106:492–500
12. Zhang Y, Xu Z, Wang H, Dong Y, Shi HN, Culley DJ, Crosby G, Marcantonio ER, Tanzi RE, Xie Z: Anesthetics isoflurane and desflurane differently affect mitochondrial function, learning, and memory. Ann Neurol 2012; 71:687–98
13. Tang JX, Baranov D, Hammond M, Shaw LM, Eckenhoff MF, Eckenhoff RG: Human Alzheimer and inflammation biomarkers after anesthesia and surgery. Anesthesiology 2011; 115:727–32
14. 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; 257:439–48
15. Price CC, Levy SA, Tanner J, Garvan C, Ward J, Akbar F, Bowers D, Rice M, Okun M: Orthopedic surgery and post-operative cognitive decline in idiopathic Parkinson's disease: Considerations from a pilot study. J Parkinsons Dis 2015; 5:893–905
16. Avidan MS, Evers AS: The fallacy of persistent postoperative cognitive decline. Anesthesiology 2016; 124:255–8
17. Mandal PK, Fodale V: Isoflurane and desflurane at clinically relevant concentrations induce amyloid beta-peptide oligomerization: An NMR study. Biochem Biophys Res Commun 2009; 379:716–20
18. Carnini A, Lear JD, Eckenhoff RG: Inhaled anesthetic modulation of amyloid beta(1-40) assembly and growth. Curr Alzheimer Res 2007; 4:233–41
19. Run X, Liang Z, Zhang L, Iqbal K, Grundke-Iqbal I, Gong CX: Anesthesia induces phosphorylation of tau. J Alzheimers Dis 2009; 16:619–26
20. Whittington RA, Bretteville A, Dickler MF, Planel E: Anesthesia and tau pathology. Prog Neuropsychopharmacol Biol Psychiatry 2013; 47:147–55
21. Yuki K, Eckenhoff RG: Mechanisms of the immunological effects of volatile anesthetics: A review. Anesth Analg 2016; 123:326–35
22. Stollings LM, Jia LJ, Tang P, Dou H, Lu B, Xu Y: Immune modulation by volatile anesthetics. Anesthesiology 2016; 125:399–411
23. Mesbah-Oskui L, Penna A, Orser BA, Horner RL: Reduced expression of α5GABAA receptors elicits autism-like alterations in EEG patterns and sleep-wake behavior. Neurotoxicol Teratol 2017; 61:115–22
24. Jounaidi Y, Cotten JF, Miller KW, Forman SA: Tethering IL2 to its receptor IL2Rβ enhances antitumor activity and expansion of natural killer NK92 cells. Cancer Res 2017; 77:5938–51
25. Goldstein PA, Eckenhoff RG: Progress on defining the molecular targets and sites of general anesthetics. Trends Pharmacol Sci 2019; 40:464–81
26. Liang L, Wei H: Dantrolene, a treatment for Alzheimer disease? Alzheimer Dis Assoc Disord 2015; 29:1–5
27. Chakroborty S, Briggs C, Miller MB, Goussakov I, Schneider C, Kim J, Wicks J, Richardson JC, Conklin V, Cameransi BG, Stutzmann GE: Stabilizing ER Ca2+ channel function as an early preventative strategy for Alzheimer's disease. PLoS One 2012; 7:e52056
28. Lin D, Feng C, Cao M, Zuo Z: Volatile anesthetics may not induce significant toxicity to human neuron-like cells. Anesth Analg 2011; 112:1194–8
29. Wang DS, Kaneshwaran K, Lei G, Mostafa F, Wang J, Lecker I, Avramescu S, Xie YF, Chan NK, Fernandez-Escobar A, Woo J, Chan D, Ramsey AJ, Sivak JM, Lee CJ, Bonin RP, Orser BA: Dexmedetomidine prevents excessive γ-aminobutyric acid type A receptor function after anesthesia. Anesthesiology 2018; 129:477–89
30. Twaroski DM, Yan Y, Zaja I, Clark E, Bosnjak ZJ, Bai X: Altered mitochondrial dynamics contributes to propofol-induced cell death in human stem cell-derived neurons. Anesthesiology 2015; 123:1067–83
31. Zhang Y, Pan C, Wu X, Dong Y, Culley DJ, Crosby G, Li T, Xie Z: Different effects of anesthetic isoflurane on caspase-3 activation and cytosol cytochrome c levels

between mice neural progenitor cells and neurons. Front Cell Neurosci 2014; 8:14
32. Whittington RA, Virág L, Gratuze M, Petry FR, Noël A, Poitras I, Truchetti G, Marcouiller F, Papon MA, El Khoury N, Wong K, Bretteville A, Morin F, Planel E: Dexmedetomidine increases tau phosphorylation under normothermic conditions *in vivo* and *in vitro*. Neurobiol Aging 2015; 36:2414–28
33. Tanaka T, Kai S, Matsuyama T, Adachi T, Fukuda K, Hirota K: General anesthetics inhibit LPS-induced IL-1β expression in glial cells. PLoS One 2013; 8:e82930
34. Yang H, Liang G, Hawkins BJ, Madesh M, Pierwola A, Wei H: Inhalational anesthetics induce cell damage by disruption of intracellular calcium homeostasis with different potencies. Anesthesiology 2008; 109:243–50
35. Ye X, Lian Q, Eckenhoff MF, Eckenhoff RG, Pan JZ: Differential general anesthetic effects on microglial cytokine expression. PLoS One 2013; 8:e52887
36. Sun Y, Zhang Y, Cheng B, Dong Y, Pan C, Li T, Xie Z: Glucose may attenuate isoflurane-induced caspase-3 activation in H4 human neuroglioma cells. Anesth Analg 2014; 119:1373–80
37. Culley DJ, Cotran EK, Karlsson E, Palanisamy A, Boyd JD, Xie Z, Crosby G: Isoflurane affects the cytoskeleton but not survival, proliferation, or synaptogenic properties of rat astrocytes *in vitro*. Br J Anaesth 2013; 110(suppl 1):i19–28
38. Zhao X, Yang Z, Liang G, Wu Z, Peng Y, Joseph DJ, Inan S, Wei H: Dual effects of isoflurane on proliferation, differentiation, and survival in human neuroprogenitor cells. Anesthesiology 2013; 118:537–49
39. Palanisamy A, Friese MB, Cotran E, Moller L, Boyd JD, Crosby G, Culley DJ: Prolonged treatment with propofol transiently impairs proliferation but not survival of rat neural progenitor cells *in vitro*. PLoS One 2016; 11:e0158058
40. Sun Y, Cheng B, Dong Y, Li T, Xie Z, Zhang Y: Time-dependent effects of anesthetic isoflurane on reactive oxygen species levels in HEK-293 cells. Brain Sci 2014; 4:311–20
41. Skelly DT, Hennessy E, Dansereau MA, Cunningham C: A systematic analysis of the peripheral and CNS effects of systemic LPS, IL-1β, [corrected] TNF-α and IL-6 challenges in C57BL/6 mice. PLoS One 2013; 8:e69123
42. Cunningham C, Campion S, Lunnon K, Murray CL, Woods JF, Deacon RM, Rawlins JN, Perry VH: Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. Biol Psychiatry 2009; 65:304–12
43. Lazic SE, Clarke-Williams CJ, Munafò MR: What exactly is 'N' in cell culture and animal experiments? PLoS Biol 2018; 16:e2005282
44. Xie Z, Dong Y, Maeda U, Alfille P, Culley DJ, Crosby G, Tanzi RE: The common inhalation anesthetic isoflurane induces apoptosis and increases amyloid beta protein levels. Anesthesiology 2006; 104:988–94
45. Wei H, Liang G, Yang H, Wang Q, Hawkins B, Madesh M, Wang S, Eckenhoff RG: The common inhalational anesthetic isoflurane induces apoptosis via activation of inositol 1,4,5-trisphosphate receptors. Anesthesiology 2008; 108:251–60
46. Zhang Y, Dong Y, Wu X, Lu Y, Xu Z, Knapp A, Yue Y, Xu T, Xie Z: The mitochondrial pathway of anesthetic isoflurane-induced apoptosis. J Biol Chem 2010; 285:4025–37
47. Wang H, Dong Y, Zhang J, Xu Z, Wang G, Swain CA, Zhang Y, Xie Z: Isoflurane induces endoplasmic reticulum stress and caspase activation through ryanodine receptors. Br J Anaesth 2014; 113:695–707
48. Zhang G, Dong Y, Zhang B, Ichinose F, Wu X, Culley DJ, Crosby G, Tanzi RE, Xie Z: Isoflurane-induced caspase-3 activation is dependent on cytosolic calcium and can be attenuated by memantine. J Neurosci 2008; 28:4551–60
49. Litman RS, Griggs SM, Dowling JJ, Riazi S: Malignant hyperthermia susceptibility and related diseases. Anesthesiology 2018; 128:159–67
50. Nagashima K, Zorumski CF, Izumi Y: Propofol inhibits long-term potentiation but not long-term depression in rat hippocampal slices. Anesthesiology 2005; 103:318–26
51. Ishizeki J, Nishikawa K, Kubo K, Saito S, Goto F: Amnestic concentrations of sevoflurane inhibit synaptic plasticity of hippocampal CA1 neurons through gamma-aminobutyric acid-mediated mechanisms. Anesthesiology 2008; 108:447–56
52. Zhou R, Bickler P: Interaction of isoflurane, tumor necrosis factor-α and β-amyloid on long-term potentiation in rat hippocampal slices. Anesth Analg 2017; 124:582–7
53. Zhan X, Fahlman CS, Bickler PE: Isoflurane neuroprotection in rat hippocampal slices decreases with aging: Changes in intracellular Ca2+ regulation and N-methyl-D-aspartate receptor-mediated Ca2+ influx. Anesthesiology 2006; 104:995–1003
54. Jevtovic-Todorovic V, Brambrick A: General anesthesia and young brain: What is new? J Neurosurg Anesthesiol 2018; 30:217–22
55. Steele LM, Sedensky MM: Approaches to anesthetic mechanisms: The *C. elegans* model. Methods Enzymol 2018; 602:133–51
56. van Swinderen B, Galifianakis A, Crowder CM: A quantitative genetic approach towards volatile anesthetic mechanisms in *C. elegans*. Toxicol Lett 1998; 100-101:309–17
57. Morgan PG, Kayser EB, Sedensky MM: *C. elegans* and volatile anesthetics. WormBook 2007: 1–11
58. Chen X, Barclay JW, Burgoyne RD, Morgan A: Using *C. elegans* to discover therapeutic compounds for

ageing-associated neurodegenerative diseases. Chem Cent J 2015; 9:65
59. Ugur B, Chen K, Bellen HJ: *Drosophila* tools and assays for the study of human diseases. Dis Model Mech 2016; 9:235–44
60. Lin M, Nash HA: Influence of general anesthetics on a specific neural pathway in *Drosophila melanogaster*. Proc Natl Acad Sci USA 1996; 93:10446–51
61. Kelz MB, Friedman E: Anesthetic sensitivity: Learning to fly. Anesthesiology 2009; 111:5–7
62. Fischer JA, Olufs ZPG, Katzenberger RJ, Wassarman DA, Perouansky M: Anesthetics influence mortality in a *Drosophila* model of blunt trauma with traumatic brain injury. Anesth Analg 2018; 126:1979–86
63. Bakalov V, Amathieu R, Triba MN, Clement MJ, Reyes Uribe L, Le Moyec L, Kaynar AM: Metabolomics with nuclear magnetic resonance spectroscopy in a *Drosophila melanogaster* model of surviving sepsis. Metabolites 2016; 6
64. Joiner WJ, Friedman EB, Hung HT, Koh K, Sowcik M, Sehgal A, Kelz MB: Genetic and anatomical basis of the barrier separating wakefulness and anesthetic-induced unresponsiveness. PLoS Genet 2013; 9:e1003605
65. Yang X, Jounaidi Y, Dai JB, Marte-Oquendo F, Halpin ES, Brown LE, Trilles R, Xu W, Daigle R, Yu B, Schaus SE, Porco JA Jr, Forman SA: High-throughput screening in larval zebrafish identifies novel potent sedative-hypnotics. Anesthesiology 2018; 129:459–76
66. Martín-Jiménez R, Campanella M, Russell C: New zebrafish models of neurodegeneration. Curr Neurol Neurosci Rep 2015; 15:33
67. 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; 29:1002–10
68. Xie Z, Culley DJ, Dong Y, Zhang G, Zhang B, Moir RD, Frosch MP, Crosby G, Tanzi RE: The common inhalation anesthetic isoflurane induces caspase activation and increases amyloid beta-protein level *in vivo*. Ann Neurol 2008; 64:618–27
69. Dong Y, Zhang G, Zhang B, Moir RD, Xia W, Marcantonio ER, Culley DJ, Crosby G, Tanzi RE, Xie Z: The common inhalational anesthetic sevoflurane induces apoptosis and increases beta-amyloid protein levels. Arch Neurol 2009; 66:620–31
70. Stratmann G, Sall JW, Bell JS, Alvi RS, May Ld, Ku B, Dowlatshahi M, Dai R, Bickler PE, Russell I, Lee MT, Hrubos MW, Chiu C: Isoflurane does not affect brain cell death, hippocampal neurogenesis, or long-term neurocognitive outcome in aged rats. Anesthesiology 2010; 112:305–15
71. Callaway JK, Jones NC, Royse AG, Royse CF: Memory impairment in rats after desflurane anesthesia is age and dose dependent. J Alzheimers Dis 2015; 44:995–1005
72. 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; 19:1245–57
73. Planel E, Bretteville A, Liu L, Virag L, Du AL, Yu WH, Dickson DW, Whittington RA, Duff KE: Acceleration and persistence of neurofibrillary pathology in a mouse model of tauopathy following anesthesia. FASEB J 2009; 23:2595–604
74. Oddo S, Caccamo A, Shepherd JD, Murphy MP, Golde TE, Kayed R, Metherate R, Mattson MP, Akbari Y, LaFerla FM: Triple-transgenic model of Alzheimer's disease with plaques and tangles: Intracellular Abeta and synaptic dysfunction. Neuron 2003; 39:409–21
75. Tang JX, Mardini F, Caltagarone BM, Garrity ST, Li RQ, Bianchi SL, Gomes O, Laferla FM, Eckenhoff RG, Eckenhoff MF: Anesthesia in presymptomatic Alzheimer's disease: A study using the triple-transgenic mouse model. Alzheimers Dement 2011; 7:521–531.e1
76. Orser BA, Wang DS: GABAA receptor theory of perioperative neurocognitive disorders. Anesthesiology 2019; 130:618–9
77. Hemmings HC Jr, Akabas MH, Goldstein PA, Trudell JR, Orser BA, Harrison NL: Emerging molecular mechanisms of general anesthetic action. Trends Pharmacol Sci 2005; 26:503–10
78. Zurek AA, Yu J, Wang DS, Haffey SC, Bridgwater EM, Penna A, Lecker I, Lei G, Chang T, Salter EW, Orser BA: Sustained increase in α5GABAA receptor function impairs memory after anesthesia. J Clin Invest 2014; 124:5437–41
79. Terrando N, Monaco C, Ma D, Foxwell BM, Feldmann M, Maze M: Tumor necrosis factor-alpha triggers a cytokine cascade yielding postoperative cognitive decline. Proc Natl Acad Sci USA 2010; 107:20518–22
80. Terrando N, Eriksson LI, Ryu JK, Yang T, Monaco C, Feldmann M, Jonsson Fagerlund M, Charo IF, Akassoglou K, Maze M: Resolving postoperative neuroinflammation and cognitive decline. Ann Neurol 2011; 70:986–95
81. Mardini F, Tang JX, Li JC, Arroliga MJ, Eckenhoff RG, Eckenhoff MF: Effects of propofol and surgery on neuropathology and cognition in the 3xTgAD Alzheimer transgenic mouse model. Br J Anaesth 2017; 119:472–80
82. Hu J, Feng X, Valdearcos M, Lutrin D, Uchida Y, Koliwad SK, Maze M: Interleukin-6 is both necessary and sufficient to produce perioperative neurocognitive disorder in mice. Br J Anaesth 2018; 120:537–45
83. 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; 55:5623–38
84. Subramaniyan S, Terrando N: Neuroinflammation and perioperative neurocognitive disorders. Anesth Analg 2019; 128:781–8
85. Safavynia SA, Goldstein PA: The role of neuroinflammation in postoperative cognitive dysfunction: Moving from hypothesis to treatment. Front Psychiatry 2018; 9:752
86. Kempuraj D, Thangavel R, Selvakumar GP, Zaheer S, Ahmed ME, Raikwar SP, Zahoor H, Saeed D, Natteru PA, Iyer S, Zaheer A: Brain and peripheral atypical inflammatory mediators potentiate neuroinflammation and neurodegeneration. Front Cell Neurosci 2017; 11:216
87. Bi J, Shan W, Luo A, Zuo Z: Critical role of matrix metallopeptidase 9 in postoperative cognitive dysfunction and age-dependent cognitive decline. Oncotarget 2017; 8:51817–29
88. Zheng B, Lai R, Li J, Zuo Z: Critical role of P2X7 receptors in the neuroinflammation and cognitive dysfunction after surgery. Brain Behav Immun 2017; 61:365–74
89. Perry VH, Cunningham C, Holmes C: Systemic infections and inflammation affect chronic neurodegeneration. Nat Rev Immunol 2007; 7:161–7
90. Xing W, Huang P, Lu Y, Zeng W, Zuo Z: Amantadine attenuates sepsis-induced cognitive dysfunction possibly not through inhibiting toll-like receptor 2. J Mol Med (Berl) 2018; 96:391–402
91. Ottens TH, Dieleman JM, Sauër AM, Peelen LM, Nierich AP, de Groot WJ, Nathoe HM, Buijsrogge MP, Kalkman CJ, van Dijk D; DExamethasone for Cardiac Surgery (DECS) Study Group: Effects of dexamethasone on cognitive decline after cardiac surgery: A randomized clinical trial. Anesthesiology 2014; 121:492–500
92. Fang Q, Qian X, An J, Wen H, Cope DK, Williams JP: Higher dose dexamethasone increases early postoperative cognitive dysfunction. J Neurosurg Anesthesiol 2014; 26:220–5
93. Valentin LS, Pereira VF, Pietrobon RS, Schmidt AP, Oses JP, Portela LV, Souza DO, Vissoci JR, Luz VF, Trintoni LM, Nielsen KC, Carmona MJ: Effects of single low dose of dexamethasone before noncardiac and nonneurologic surgery and general anesthesia on postoperative cognitive dysfunction-A phase III double blind, randomized clinical trial. PLoS One 2016; 11:e0152308
94. Glumac S, Kardum G, Sodic L, Supe-Domic D, Karanovic N: Effects of dexamethasone on early cognitive decline after cardiac surgery: A randomised controlled trial. Eur J Anaesthesiol 2017; 34:776–84
95. Zhu Y, Yao R, Li Y, Wu C, Heng L, Zhou M, Yan L, Deng Y, Zhang Z, Ping L, Wu Y, Wang S, Wang L: Protective effect of celecoxib on early postoperative cognitive dysfunction in geriatric patients. Front Neurol 2018; 9:633
96. Terrando N, Gómez-Galán M, Yang T, Carlström M, Gustavsson D, Harding RE, Lindskog M, Eriksson LI: Aspirin-triggered resolvin D1 prevents surgery-induced cognitive decline. FASEB J 2013; 27:3564–71
97. Yang T, Xu G, Newton PT, Chagin AS, Mkrtchian S, Carlström M, Zhang XM, Harris RA, Cooter M, Berger M, Maddipati KR, Akassoglou K, Terrando N: Maresin 1 attenuates neuroinflammation in a mouse model of perioperative neurocognitive disorders. Br J Anaesth 2019; 122:350–60
98. Huffman WJ, Subramaniyan S, Rodriguiz RM, Wetsel WC, Grill WM, Terrando N: Modulation of neuroinflammation and memory dysfunction using percutaneous vagus nerve stimulation in mice. Brain Stimul 2019; 12:19–29
99. Adlard PA, Perreau VM, Pop V, Cotman CW: Voluntary exercise decreases amyloid load in a transgenic model of Alzheimer's disease. J Neurosci 2005; 25:4217–21
100. Prado Lima MG, Schimidt HL, Garcia A, Daré LR, Carpes FP, Izquierdo I, Mello-Carpes PB: Environmental enrichment and exercise are better than social enrichment to reduce memory deficits in amyloid beta neurotoxicity. Proc Natl Acad Sci USA 2018; 115:E2403–9
101. Kawano T, Eguchi S, Iwata H, Tamura T, Kumagai N, Yokoyama M: Impact of preoperative environmental enrichment on prevention of development of cognitive impairment following abdominal surgery in a rat model. Anesthesiology 2015; 123:160–70
102. Fan D, Li J, Zheng B, Hua L, Zuo Z: Enriched environment attenuates surgery-induced impairment of learning, memory, and neurogenesis possibly by preserving BDNF expression. Mol Neurobiol 2016; 53:344–54
103. Feng X, Uchida Y, Koch L, Britton S, Hu J, Lutrin D, Maze M: Exercise prevents enhanced postoperative neuroinflammation and cognitive decline and rectifies the gut microbiome in a rat model of metabolic syndrome. Front Immunol 2017; 8:1768
104. Kilkenny C, Browne WJ, Cuthill IC, Emerson M, Altman DG: Improving bioscience research reporting: The ARRIVE guidelines for reporting animal research. PLoS Biol 2010; 8:e1000412
105. Fergusson DA, Avey MT, Barron CC, Bocock M, Biefer KE, Boet S, Bourque SL, Conic I, Chen K, Dong YY, Fox GM, George RB, Goldenberg NM, Gragasin FS, Harsha P, Hong PJ, James TE, Larrigan SM, MacNeil JL, Manuel CA, Maximos S, Mazer D, Mittal R, McGinn R, Nguyen LH, Patel A, Richebé P, Saha TK, Steinberg BE, Sampson SD, Stewart DJ, Syed S, Vella K, Wesch NL, Lalu MM; Canadian Perioperative Anesthesia Clinical Trials Group: Reporting preclinical anesthesia study (REPEAT):

Evaluating the quality of reporting in the preclinical anesthesiology literature. PLoS One 2019; 14:e0215221
106. Sparkman NL, Johnson RW: Neuroinflammation associated with aging sensitizes the brain to the effects of infection or stress. Neuroimmunomodulation 2008; 15:323–30
107. Prenderville JA, Kennedy PJ, Dinan TG, Cryan JF: Adding fuel to the fire: The impact of stress on the ageing brain. Trends Neurosci 2015; 38:13–25
108. Eckenhoff MF, Cunningham C: Animal Models and Cognitive Testing of Perioperative Neurocognitive Disorder, The Perioperative Neurocognitive Disorders. Edited by Eckenhoff RGT, N. Cambridge, England, Cambridge University Press, 2019, pp 61–81
109. Mackensen GB, Sato Y, Nellgård B, Pineda J, Newman MF, Warner DS, Grocott HP: Cardiopulmonary bypass induces neurologic and neurocognitive dysfunction in the rat. Anesthesiology 2001; 95:1485–91
110. Liang P, Shan W, Zuo Z: Perioperative use of cefazolin ameliorates postoperative cognitive dysfunction but induces gut inflammation in mice. J Neuroinflammation 2018; 15:235
111. Murray C, Sanderson DJ, Barkus C, Deacon RM, Rawlins JN, Bannerman DM, Cunningham C: Systemic inflammation induces acute working memory deficits in the primed brain: Relevance for delirium. Neurobiol Aging 2012; 33:603–616.e3
112. Ren Q, Peng M, Dong Y, Zhang Y, Chen M, Yin N, Marcantonio ER, Xie Z: Surgery plus anesthesia induces loss of attention in mice. Front Cell Neurosci 2015; 9:346
113. Chesler EJ, Wilson SG, Lariviere WR, Rodriguez-Zas SL, Mogil JS: Influences of laboratory environment on behavior. Nat Neurosci 2002; 5:1101–2
114. Charan J, Kantharia ND: How to calculate sample size in animal studies? J Pharmacol Pharmacother 2013; 4:303–6
115. Aban IB, George B: Statistical considerations for preclinical studies. Exp Neurol 2015; 270:82–7
116. Cressey D: UK funders demand strong statistics for animal studies. Nature 2015; 520:271–2
117. Collins FS, Tabak LA: Policy: NIH plans to enhance reproducibility. Nature 2014; 505:612–3
118. Neumann K, Grittner U, Piper SK, Rex A, Florez-Vargas O, Karystianis G, Schneider A, Wellwood I, Siegerink B, Ioannidis JP, Kimmelman J, Dirnagl U: Increasing efficiency of preclinical research by group sequential designs. PLoS Biol 2017; 15:e2001307
119. Laajala TD, Jumppanen M, Huhtaniemi R, Fey V, Kaur A, Knuuttila M, Aho E, Oksala R, Westermarck J, Mäkelä S, Poutanen M, Aittokallio T: Optimized design and analysis of preclinical intervention studies *in vivo*. Sci Rep 2016; 6:30723