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Perioperative Neurocognitive Disorder: State of the Preclinical Science

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Summary

The purpose of this white paper is to provide a succinct summary of the different experimental approaches that have been used in preclinical post-operative 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 post-operative cognitive dysfunction, and to identify knowledge gaps to be filled by further research. We 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, our goal is to improve the quality and consistency of

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post-operative cognitive dysfunction /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, we conclude with general guidelines for designing, conducting and reporting perioperative neurocognitive disorder rodent research.

Introduction

Patients over the age of about 65 are the largest consumer of procedural care.[1] Impairments in cognitive ability are the most common complications experienced in the post-operative period by these older individuals.[2,3] These impairments include post-operative 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 re-emerges.[4,5] Previously, all forms of impairment were called ‘post-operative cognitive dysfunction’ (POCD), but more recently, a recommended change to the ‘Perioperative Neurocognitive Disorders (PND)’ 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 (DSM-5), such as Alzheimer’s disease (AD)[8–14] and Parkinson’s disease.[15] Clinical studies have identified age, infection and pre-existing cognitive disorders as consistent risk factors for PND;[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., post-operative delirium), the relationship of post-operative cognitive impairment with the surgery or anesthetic itself remains uncertain. Thus, despite consensus on the existence and character of PND, 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 PND 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 PND, and to identify knowledge gaps that need to be 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 PND 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 PND

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 biological variation but generally reflects only technical variation. Examples here were the demonstration that some general anesthetics accelerate the aggregation of the AD associated amyloid β[8,17] protein, through a defined biophysical mechanism.[18] Given the phenotypic similarity between AD and some forms of PND, these studies set the stage for discussion below on cell and animal studies, 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 (DAMPs), 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 PND 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, inter-molecular 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 (NMDA) receptors, hyperpolarization-activated cyclic nucleotide–gated

(HCN) channels, tandem-pore potassium (K2P) channels, transient receptor potential (TRPx), etc) 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 ($InsP_3R$), and the ryanodine receptors (RyR) 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, an RyR inhibitor, is being investigated as a therapy for neurodegeneration,[26,27] but has not yet been explored in intact animal models of POCD/PND. 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 $GABA_AR$) has translated to produce something like delayed neurocognitive recovery (dNCR), a diagnosis under the new PND terminology (see below under 'Rodents).

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^{o}C) could be three to five fold higher than those achieved at physiological temperature (37^{o}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 co-solvents or emulsifiers as in the case 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 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 PND. [10,28–40] Stable cell lines (e.g. CHO, HEK, 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 3-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, biological 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; 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 physiological disruption (hypoventilation, hypotension, etc) which is challenging to mimic in isolated cells.

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

Fifth, as noted above, it is challenging to mimic surgery, although stimulation with DAMPs, cytokines, chemokines or lipopolysaccharide (LPS) 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 (2011–2016), it was revealed that only 22% of studies used replicates correctly.[43] Researchers need to distinguish between biological, 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 biological ‘n’ in primary culture will refer to the actual number of animals (if not pooled) from which the cell were isolated. Electrophysiological 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 either 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 POCD/PND 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 RyR,[49] and dexmedetomidine prevents the overexpression of α5-containing $GABA_A$ receptors on the neuronal surface.[29]

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 (LTP) of synapses,[50–52] a cellular surrogate for memory and learning, which is possibly disrupted in POCD/PND. 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 biological variability. Other limitations include reduced viability of slices from aged animals,[53] challenges in assessing neurogenesis, and that cell-cell connections, especially long range ones that might be most influenced by anesthetics, are invariably disrupted. Moreover, many cells are damaged, 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 POCD/PND

Many animal models that range from worms to non-human 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 have been published over the past decade, and this body of work is reviewed in detail elsewhere.[54] When referring to PND/POCD, we focus on the effect of anesthesia and surgery on the aging animal brain specifically. No studies of PND/POCD in non-human 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 IACUC concerns), clear and consistent behavior, completely sequenced genome, transparency and structurally understood nervous system. It is also sensitive to all general anesthetics, although about 5–10 fold 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. POCD/PND 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's Disease,[58] it has not received much attention in the POCD/PND 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/phenotype relationship. Much work has been conducted in the neurodegeneration pathways,[59] but again, little in the POCD/PND 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 prior and ongoing work in neurodegeneration, sepsis and anesthesia, it seems that an opportunity to study POCD/PND exists in the fly.

Danio rerio (Zebrafish)

Similar to the above, 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 POCD/PND 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, this requires that protocols involving fish be approved by IACUCs. 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-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 POCD/PND 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 POCD/PND 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 POCD/PND, researchers included other stresses or vulnerabilities. For example, recent studies have included aged rodents, typically 18–24 months of age. In older animals, post-anesthesia behavioral decrements tend to be larger and more durable.[9,70,71] Moreover, since many patients come to surgery with pre-existing cognitive impairments, and since wild type rodents tend not to suffer from anything resembling Alzheimer's disease, researchers have begun to repeat their studies with transgenic animals that include human Alzheimer's 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 above 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 dNCR.[78] Specific antagonists of the α5 $GABA_AR$ 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 PND/POCD.

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, biochemical evidence of neuroinflammation and a

greater decrement in the behavior. When age, a genetic vulnerability, a co-morbidity, and surgery were all combined in the study design, the decrements in behavior became much more durable (>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, the presence of pre-existing vulnerabilities (e.g., age, genetic and co-morbidities), the large multifactorial stress of the surgery amplifies any ongoing CNS inflammation or injury, a process potentially modulated by other drugs like anesthetics.

Evidence suggests that neuroinflammation after surgery plays a key role in POCD/PND.[84,85] The pre-existing vulnerabilities mentioned above are thought to increase blood brain barrier (BBB) permeability,[86] and allow the peripheral innate immune molecules, generated by surgical tissue damage, to enter the CNS to further enhance neuroinflammation and injury. Moreover, mice that lack genes to mount significant neuroinflammation did not develop POCD after anesthesia and surgery.[87,88] Even in the absence of surgery, stimulation of the innate immune response by lipopolysaccharide (LPS), or inducing sepsis, produces transient decrements in behavioral assays, reminiscent of dNCR, or "sickness behavior."[89,90] Blockage of either TNFα or IL-6 using antibodies, effectively reduced rodent POCD, but also delayed wound healing.[79,82] More conventional anti-inflammatory drugs (dexamethasone/cox-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 pro-resolution 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's or Prion Disease, or on preexisting cerebrovascular disease, have not been reported, despite these disorders being fairly common in aged surgical populations. Similarly, traumatic brain injury (TBI) is thought to enhance vulnerability to Alzheimer's and Parkinson's disease. The effect of anesthesia and surgery on PND/POCD in humans or animals with a history of even mild TBI has not been reported. Moreover, an association between depression, 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 non-pharmacological approaches is suggested by evidence showing that environmental enrichment slows cognitive decline in a murine Alzheimer disease model, [99,100] as well as POCD in rodents [101,102] Growing evidence also implicates the gut microbiome in several neuroinflammatory models and its contribution in PND is just beginning to be explored.[103]

Suggestions for Rodent PND/POCD research

The hundreds of rodent studies of PND/POCD 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., ARRIVE (Animal Research: Reporting of in vivo Experiments)).[104] It should be noted that even 4–6 years after publication of the ARRIVE 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 'POCD' has been used in the *clinical* literature to refer to any post-operative 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 'POCD.'[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 post-operatively, with full apparent recovery thereafter. This might parenthetically be termed, dNCR, even though the required "cognitive concern" cannot be voiced. In another study where the deficits appeared to be persistent even to 3 months post-operatively, this might be 'neurocognitive disorder' (NCD), 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' NCD. Ultimately, however, researchers simply need to be precise about what they did and why when reporting.

Animal age, sex and environment

PND/POCD 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 PND/POCD mechanisms and influences should include animals aged to at least 70–80% 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 biological variable that must be considered in PND/POCD research. Delirium and cognitive impairment is 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, pre-existing cognitive decline is a major risk factor for PND/POCD, 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 PND/POCD 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 physiological 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 that 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 (EEG) monitoring of aged animals undergoing anesthesia has not been reported in POCD/PND studies, but might be considered since the anesthetic sensitivity of genetically altered animals is rarely determined before using them in such studies. In addition, EEG 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. ARRIVE 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, physiological monitoring, and translational relevance.

Surgical procedure

Reported surgical procedures in rodents have varied, and have included simple skin incision/vascular exposure, splenectomy, cecectomy/appendectomy, partial hepatectomy, and tibial fracture[108]. Other models exist but have not been explored for PND, such as cardiopulmonary bypass.[109] All have merit, as POCD 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 PND/POCD.[110]

Behavioral assays

The earliest form of PND to occur in the human after anesthesia and surgery is post-operative delirium (POD) 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/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 PND/POCD we can offer the following suggestions. Since human PND/POCD 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, vision, etc), on which many behavioral assays are critically dependent; baseline values and control groups are necessary. Evidence suggests that environment, including the researchers themselves,[113] influence 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 5 or 6 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 biological 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 biological 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 seconday 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 analysis of variance (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, non-parametric tests must be considered to avoid misleading results. When multiple independent tests are planned, with no a priori focus, such as is common in 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% confidence interval in order to gauge translational relevance. Finally, as outlined in the ARRIVE 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 biological differences.[116,117] Further, critical biological variables like age, sex and co-morbidities 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 PND/POCD 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 pre-existing neuronal vulnerabilities other than Alzheimer disease, such as Parkinson's disease and TBI? 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 PND/POCD. 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. The latter deserves 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.

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Summary Statement

A bewildering variety of preclinical post-operative cognitive dysfunction studies has been reported over the last two decades. Herein we succinctly summarize the approaches and models, some of the accrued knowledge, and suggestions for future design and reporting.

Table 1.

PND knowledge gaps potentially addressable in preclinical studies.

Which animal model (species, strain, surgery, anesthesia, etc.) best reproduces the clinical phenotype of each form of PND (delirium, dNCR and NCD)?
Should the known risk factors for clinical PND (age, cognitive impairment, frailty, etc.) be added to the animal model (#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 pro-inflammatory versus pro-resolving 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 (ROI) 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, diet, etc.) influence it?
Is pre-existing 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?