HHS Public Access

Author manuscript

Alzheimers Dement. Author manuscript; available in PMC 2023 October 14.

Published in final edited form as:

Alzheimers Dement. 2023 May ; 19(5): 2150–2174. doi:10.1002/alz.12941.

Preclinical and translational models for delirium: Recommendations for future research from the NIDUS delirium network

Sarinnapha M. Vasunilashorn[1,2,3], **Nadia Lunardi**[4], **John C. Newman**[5,6], **Gregory Crosby**[2,7], **Leah Acker**[8], **Ted Abel**[9], **Seema Bhatnagar**[10,11], **Colm Cunningham**[12,13], **Rafael de Cabo**[14], **Laura Dugan**[15,16,17], **Joseph A. Hippensteel**[18], **Yumiko Ishizawa**[2,19], **Shouri Lahiri**[20], **Edward R. Marcantonio**[1,2,21,22], **Zhongcong Xie**[2,19], **Sharon K. Inouye**[2,21,22], **Niccolò Terrando**[23,24,25,26], **Roderic G. Eckenhoff**[11],

NIDUS Delirium Network

[1]Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA

[2]Harvard Medical School, Boston, Massachusetts, USA

[3]Department of Epidemiology, Harvard T. H. Chan School of Public Health, Boston, Massachusetts, USA

[4]Department of Anesthesiology, University of Virginia, Charlottesville, Virginia, USA

[5]Department of Medicine, University of California, San Francisco, California, USA

[6]Buck Institute for Research on Aging, Novato, California, USA

[7]Department of Anesthesiology, Brigham and Women's Hospital, Boston, Massachusetts, USA

[8]Department of Anesthesiology, Duke University, Durham, Massachusetts, USA

[9]Department of Neuroscience and Pharmacology, Carver College of Medicine, University of Iowa, Iowa City, Iowa, USA

[10]Department of Anesthesiology and Critical Care, Children's Hospital of Philadelphia, Philadelphia, Pennsylvania, USA

[11]Department of Anesthesiology and Critical Care, University of Pennsylvania, Perelman School of Medicine, Philadelphia, Pennsylvania, USA

[12]School of Biochemistry and Immunology, Trinity Biomedical Sciences Institute, Dublin, Ireland

[13]Trinity College Institute of Neuroscience, Trinity College, Dublin, Ireland

Correspondence: Sarinnapha M. Vasunilashorn, Division of General Medicine; Beth Israel Deaconess Medical Center, 330 Brookline Ave, CO-205, Boston, MA 02215, USA. svasunil@bidmc.harvard.edu.
Sarinnapha M. Vasunilashorn and Nadia Lunardi are co-first authors.
Sharon K. Inouye, Niccolò Terrando, and Roderic G. Eckenhoff are co-senior authors.

CONFLICTS OF INTEREST

All authors report no conflicts of interest. Author disclosures are available in the supporting information.

[14]Translational Gerontology Branch, Intramural Research Program, National Institute on Aging, Baltimore, Maryland, USA

[15]Vanderbilt Brain Institute, Vanderbilt University, Nashville, Tennessee, USA

[16]Division of Geriatric Medicine, Department of Medicine, Vanderbilt University Medical Center, Nashville, Tennessee, USA

[17]VA Tennessee Valley Geriatric Research, Education, and Clinical Center (GRECC), Nashville, Tennessee, USA

[18]Department of Medicine, University of Colorado Anschutz Medical Campus, Aurora, Colorado, USA

[19]Department of Anesthesia, Critical Care and Pain Medicine, Massachusetts General Hospital, Boston, Massachusetts, USA

[20]Department of Neurology, Neurosurgery, and Biomedical Sciences, Cedar-Sinai Medical Center, Los Angeles, California, USA

[21]Department of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA

[22]Marcus Institute for Aging Research, Hebrew SeniorLife, Boston, Massachusetts, USA

[23]Department of Anesthesiology, Duke University, Durham, North Carolina, USA

[24]Department of Cell Biology, Duke University, Durham, North Carolina, USA

[25]Department of Immunology, Duke University, Durham, North Carolina, USA

[26]Duke Center for the Study of Aging and Human Development, Duke University School of Medicine, Durham, USA

Abstract

Delirium is a common, morbid, and costly syndrome that is closely linked to Alzheimer's disease (AD) and AD-related dementias (ADRD) as a risk factor and outcome. Human studies of delirium have advanced our knowledge of delirium incidence and prevalence, risk factors, biomarkers, outcomes, prevention, and management. However, understanding of delirium neurobiology remains limited. Preclinical and translational models for delirium, while challenging to develop, could advance our knowledge of delirium neurobiology and inform the development of new prevention and treatment approaches. We discuss the use of preclinical and translational animal models in delirium, focusing on (1) a review of current animal models, (2) challenges and strategies for replicating elements of human delirium in animals, and (3) the utility of biofluid, neurophysiology, and neuroimaging translational markers in animals. We conclude with recommendations for the development and validation of preclinical and translational models for delirium, with the goal of advancing awareness in this important field.

Keywords

animal models; biomarkers; delirium

1 | INTRODUCTION

What Is Delirium?

Delirium is an acute confusional state that is common, morbid, and costly. It is characterized by both an acute-onset and fluctuating nature that involves cognitive disturbances (eg, inattention, an altered level of consciousness, and disordered thinking) and noncognitive features (eg, altered sleep-wake cycles). Primary features include (1) a reduced awareness of the surrounding environment (eg, difficulty focusing and/or often changing topics; being easily distracted; withdrawal or absence of response to surroundings), (2) poor thinking skills (eg, not knowing where or who others are, forgetting recent events, rambling or nonsense speech), and (3) behavioral and emotional changes (eg, anxiety, fear or distrust; restlessness; being quiet or withdrawn). Symptoms of delirium typically begin suddenly (eg, within a few hours to days) and can often come and go throughout the day. Moreover, signs of delirium often worsen in unfamiliar settings (eg, hospital, intensive care unit [ICU], nursing home).

Delirium is currently characterized by three major subtypes: (1) the more obvious hyperactive type, (2) the frequently missed, though more common, hypoactive type, and (3) a combination of hyperactive and hypoactive types seen in mixed delirium. Hyperactive delirium includes behaviors that range from restlessness to agitation and constant movement.[1,2] Hypoactive delirium is characterized by at least one of the following: paucity of speech with or without prompting, slow or no movement, or unresponsiveness.[1] Mixed delirium can present as switching back and forth between restlessness (a hyperactive feature) and sluggishness (a hypoactive feature). This broad phenomenological range, coupled with the fluctuating course of delirium, makes this syndrome particularly challenging to identify and determine its underlying pathophysiological mechanisms.

Delirium occurs in ≈20% of older patients admitted to a general medicine ward, >20% of high-risk patients following major surgery, and 50% to 70% of mechanically ventilated patients in critical care settings.[3] Delirium is not only emotionally disturbing to patients, families, and caregivers, but it is strongly associated with many adverse outcomes, including longer length of hospital stay, greater morbidity and mortality, higher rates of institutionalization, and increased risk of long-term cognitive decline and dementia.[4–7] Given its association with increased health care utilization and costs, delirium holds substantial implications for public health and health policy. For example, developing delirium during an intensive care unit (ICU) stay was associated with a 39% increase in the cost of ICU care. More recent research has shown 30-day cumulative incremental costs attributable to ICU delirium upwards of $22,000 per patient. It is estimated that total direct 1-year U.S. health care costs attributable to delirium range from $143 to $152 billion for all older persons and from $26 to $42 billion per year in older surgical patients,[8] costs rivaling those associated with cancer and diabetes.

Given the associated poor outcomes and increased costs attributed to delirium, the imperative for delirium mitigation is pronounced. With empirical studies suggesting the partial effectiveness of nonpharmacological delirium mitigation strategies and given the

established epidemiological link between delirium and dementia (eg, potential acceleration of dementia following an episode of delirium), delirium may be a preventable contributor to the increasing burden of dementia. This hypothesis has been highlighted via recent calls for a public health campaign to mitigate delirium as a means of reducing the global burden of dementia.[9]

What Is Known about the Biological Basis of Delirium?

In recent decades, clinical studies of delirium have advanced our knowledge in terms of its incidence and prevalence, risk factors, biomarkers, outcomes, prevention, and management, topics that have been comprehensively reviewed in recent work.[3,7,10–13] However, the fundamental understanding of delirium neurobiology remains unclear. Several well-studied areas showing promising associations with delirium include neuroinflammation, neuronal injury, and neurotransmitter imbalance.[3,14–23] To more fully understand the complex neurobiological underpinnings of delirium, it will be necessary to identify causal mechanisms. To determine such causal mechanisms, experimental studies in animal models provide the most common and compelling approach. Although this is a developing area, high-quality science in this area will help advance our understanding of the fundamental neurobiology of this clinical syndrome. Table 1 presents key conceptual goals for the use of animal models in delirium research and includes some practical examples of experimental questions.

1.1 | Benefits of using animal models in delirium research

Multifactorial contributors to delirium have been identified in human studies, including both vulnerability and precipitating factors.[10] Animal models allow investigators to carefully consider these individual factors to rigorously test mechanistic hypotheses and develop potential interventions and treatments. Notably, animal models have been used extensively for preclinical testing of many human treatments, such as for Alzheimer's disease (AD) and AD-related dementias (AD/ADRD),[11] Parkinson's disease,[14] multiple sclerosis,[15] and heart failure.[16] Although progress in these areas has been slow and plagued with setbacks, it is relevant to note that the only currently approved disease-modifying therapy for AD/ADRD has been based on decades of animal model research.[17] Importantly, since AD/ADRD and mild cognitive impairment (MCI) are strong risk factors for human delirium, the availability of multiple rodent models of different aspects of these neurodegenerative disorders could be leveraged to enhance the validation of studies focused on delirium phenotypes.

The use of animal models to observe disease-associated motor neuron degeneration, for which brain tissue in humans is rarely available, has informed the development of therapeutic interventions for amyotrophic lateral sclerosis.[18] In this paper, we adopt the definition of a potential biomarker given by the National Institutes of Health (NIH) Biomarkers Definitions Working Group panel: a "characteristic that is objectively measured and evaluated as an indicator of normal biological processes, pathogenic processes, or pharmacological responses to a therapeutic intervention."[24] Here we use the term biomarker as an indicator of a pathogenic processes pertaining to delirium.

To guide the future development of animal models for delirium and enhance understanding of delirium neurobiology, the Network for the Investigation of Delirium: Unifying Scientists (NIDUS) assembled a group of scientific investigators actively working in the field of delirium and/or animal models with expert knowledge from varied settings in which delirium occurs (eg, medical, surgical, and ICU). A major goal of the NIH-funded NIDUS is to identify research priorities for the field.[25] Such studies are conducted on a voluntary basis and have followed a standard format in identifying experts across disciplines, proposing areas to be addressed, forming writing groups, reviewing relevant literature, and drafting summaries and recommendations. During 2020, the development of animal models for delirium was identified by NIDUS as a research priority for the field.[25] To advance a proposed paper, a core group of NIDUS members (SMV, NL, JN, GC, SKI, NT, RGE) met virtually five times from October 2020 to March 2021 (October 30, November 4, and December 15, 2020, and February 2 and March 2, 2021) to discuss (1) the current state of knowledge of animal models of delirium (JN, GC, JAH), (2) the development of animal models of delirium, including general principles and realistic goals (TA, SB, RGE), (3) phenotypic and behavioral assessments of delirium features in animal models (NL, LD, YI, NT), and (4) approaches to validating preclinical models of delirium (TA, SB, RGE). Subgroups were formed to address each of these four topics and met separately for one to three sessions each to further develop and refine each of these points (individual membership of each of the aforementioned subgroups). The subsections were combined into a single document, with introductory sections and tables drafted as needed by the core group. Additional members provided critical feedback on drafts of the manuscript (LA, CC, RdC, SL, ERM, ZX). Consensus was achieved through an iterative feedback process with all members of the group.

It is important to acknowledge that we are at an early stage in the development of animal model research in delirium. We had hoped that this expert consensus paper might provide preliminary recommendations for the field to move forward and to incorporate best practices and lessons learned from other fields, such as AD and aging. Since our goal was to provide preliminary research recommendations for a developing field and not to develop more advanced guidelines at this early stage, elements of the Grading of Recommendations, Assessment, Development and Evaluations (GRADE) process, such as input from pharmaceutical industry personnel, funding agencies, laypersons, and patients, were considered outside the scope of this project.

1.2 | Validation of preclinical models of delirium

A recurring theme in this work is that no animal model perfectly recapitulates all aspects of a human condition, especially a complex behavioral syndrome like delirium, but that validity needs to be considered in the context of the specific experimental goal. Common model validity frameworks include face validity, construct validity, predictive validity, and target validity[26] (see Table 2 for applications to animal models). For example, a delirium model would have high face validity if the animal demonstrated a recognizable delirium-like phenotype (detailed in what follows) and high construct validity if this occurred in a context associated with delirium in humans and involving suspected elements of human delirium pathophysiology. Examples of delirium models that combine reasonable

face and construct validity are sepsis, general anesthesia, and anesthesia/surgery models. Other elements of construct validity are not yet available for delirium. There are currently no well-established genetic predispositions (eg, candidate genetic markers) that could be adapted to create genetic models for delirium, as has been productively utilized for AD/ADRD. Although there are no definitive biomarkers for delirium, several promising markers have been identified (see Biomarkers section in what follows). In addition, there are no definitive objective measures of delirium in rodents, although this is an area of highly active investigation.

Predictive validity is the ability to predict outcomes related to delirium, such as clinical outcomes or the response to targeted interventions. Rodent models of depression may have uncertain face validity, but the response to human antidepressants provides a useful degree of predictive validity.[27] Delirium currently lacks pharmacological therapies, although as mentioned earlier, nonpharmacological interventions to prevent delirium may provide an as-yet underexplored means to test the predictive validity of delirium animal models. Finally, target validity is the impact on specific pathophysiological pathways known to be associated with the condition. This is currently not readily testable in delirium since the critical neurobiological or pathological pathways have not been defined and could vary between species. Many mechanistic contributors to, and mediators of, delirium have been hypothesized, with perhaps the strongest clinical support for central nervous system inflammatory activation, but overall the specific molecular neurobiology of delirium remains unresolved. Validation of delirium animal models is an ongoing, iterative process that should incorporate as many of these frameworks as possible that become testable over time.

1.3 | Animal models of delirium: Current state of development

To date, most efforts to model delirium in laboratory animals have used a framework of an underlying vulnerability with a superimposed acute precipitating factor, a framework that has also informed the earliest systematic understanding of human delirium[10] and that has been applied to other multifactorial syndromes of older adults such as frailty or falls.[28,29] Table 3 lists the vulnerabilities and precipitants reported thus far in preclinical models for delirium. Part of the challenge of modeling delirium is the complexity of the clinical syndrome with its multifactorial etiology, both physiological and environmental. As in many manifestations of brain function, both normal and abnormal, delirium can best be considered an emergent behavior of a complex system.[30] The heterogeneity among the vulnerability and precipitating factors likely reflects the multifactorial nature of delirium and must be considered when modeling a complex clinical syndrome such as delirium. Models based on a single intervention, such as apolipoprotein E (APOE) gene knockout, mutant APP transgene overexpression, or high fat diet feeding, may not be as relevant because a single intervention is unlikely to prevent or substantially improve adverse outcomes associated with the condition.

1.3.1 | Vulnerability factors—Because aging and neurodegenerative disease are primary nonmodifiable risk factors for human delirium, they have often been employed as vulnerability factors in rodent models. Wild-type (WT) mice or rats are aged up to 24 months (approximate median lifespan) for aging-related research.[31–38] Neurodegenerative

models used in the delirium literature include the ME7 prion model,[39–42] APPSwDI/mNos2$^{-/-}$ AD model,[43] and murine-p75-saporin immunotoxin (mu-p75-sap) to induce selective lesions of the basal forebrain cholinergic system in mice.[23] Many other neurodegeneration models exist, and much work remains to integrate the diversity of available models to better understand the mechanisms that predispose a person to delirium and dementia. It is also important to note that vulnerability factors are not necessarily required in animal models, as delirium has sometimes been observed in their absence, particularly when the precipitant is sufficiently severe (eg, sepsis).

1.3.2 | Precipitating factors—Superimposed on any baseline vulnerabilities are precipitating factors that attempt to mimic common acute events or stresses that lead to delirium in humans. This can take either a reductionist or emulative approach, depending on the experimental goals. An emulative approach attempts to mimic the pathology of delirium by simulating a relevant multifactorial clinical scenario. Models used include experimental tibia fracture and repair under anesthesia and analgesia;[43–45] anesthesia and laparotomy;[36,38,46,47] mechanical ventilation-induced acute lung injury, *Escherichia coli* urinary tract infection, intratracheal administration of *Streptococcus pneumoniae* to cause pneumonia, sepsis, and multiorgan failure;[48] cardiopulmonary bypass under anesthesia with hypothermic cardiac arrest,[49] and the combination of anesthesia, laparotomy, and simulated ICU environment.[35] The reductionist approach adopts a more specific precipitating factor, usually in the context of mechanistic studies involving that factor. Examples include bacterial lipopolysaccharide (LPS),[32–34,39–41,50,51] interleukin (IL)-1β,[41] intracerebroventricular Aβ oligomer injection,[55] and intracerebroventricular albumin infusion.[31] Caution with interpretation should be exercised in that some of these precipitants, LPS in particular, produce physiological changes that are independently associated with delirium, such as hypoglycemia[41] and localized cerebral hypoxia.[52]

Many of the elements that have been implicated as contributors to or precipitants of delirium (eg, polypharmacy,[53,54] sleep disruption[32,50,55]) and that inform the core of effective multicomponent delirium prevention interventions have not yet been specifically studied in a delirium preclinical context. Other delirium-associated elements ready for investigation include exacerbating factors (eg, frailty, hypoxia, sensory deprivation, dehydration, and social isolation) and protective factors (eg, mobility, exercise, nutrition, cognitive stimulation, and socialization).

1.4 | Limitations of preclinical models for delirium and next steps for development

1.4.1 | General principles—First, it is irrational to expect that animals will experience the full complexity or exhibit the characteristics of delirium that are well documented in humans.[56] Second, like many neuropsychiatric disorders, the constellation of features present in the human syndrome of delirium are not easily quantifiable or disentangled in small animals (eg, inattention, disorientation, hallucinations), though these are individually nondiagnostic. Given these challenges, modeling of delirium in preclinical models requires recreating clinically known risk factors, for example advanced age, inflammation, or neurodegeneration, with clearly defined behavioral endpoints. To the extent that these behaviors reflect those of human delirium, the underlying neurobiology can be explored.

As previously stated, it is unrealistic to expect any animal model to recapitulate all required features of the clinical diagnosis of delirium. Without knowing the underlying neurophysiology, the best we can currently do is develop a set of animal models with individual cognitive and behavioral features resembling human delirium, and then dissect and explore the common biological mechanisms. This distinction between an animal model with individual features of human delirium versus recapitulating the full repertoire of features of human delirium in animals is important, but it does not negate the utility of animal models. We are very limited in our ability to determine the underlying neurobiology of delirium in humans, so animal models are necessary in order to explore mechanisms and to discover and test therapeutic targets and/or interventions for delirium and its associated adverse outcomes.

We acknowledge up front that a major challenge for future work in the development of preclinical models will be needed to determine the best approach to capture delirium in phenotypic and behavioral tests. Many questions remain unanswered at this time: How do we integrate individual tests in a battery? How do we account for key factors such as timing and sequence? And how should the data be aggregated and analyzed? These questions underscore the pressing need to advance methodological approaches to move the field forward.

1.4.2 | Realistic goals for preclinical models—Rodents, and particularly in-bred laboratory mice, are the most common preclinical model for many diseases and disorders. Examples abound, from depression and schizophrenia to atherosclerotic coronary disease and AD/ADRD. Despite this, few of these syndromic diseases map precisely from human to mouse.[57] Thus, we recommend a cautious approach that considers the limitations of developing preclinical models for delirium.

Several potential approaches for the creation of preclinical models were considered. The first approach uses knowledge of the genetic basis of human disease to create genetically modified mice. This has included knocking out the low-density lipoprotein (LDL) receptor or apoE lipoprotein to create models of hypercholesterolemia-driven atherosclerosis[58] or overexpressing APP transgenes with human familial AD mutations to create models of AD.[59] Such approaches are currently unavailable for delirium, which is unlikely to have a monogenic inherited predisposition.

A second approach, available in cases where mouse physiology is similar to that of humans, involves mimicking the conditions that elicit the human disease. For example, investigators have used high-fat/high-sugar feeding of sedentary mice to mimic many of the features of human obesity, type 2 diabetes, and nonalcoholic fatty liver disease.[60] In delirium research, this approach is being explored by subjecting mice to surgery with anesthesia, systemic infections, or inflammatory processes, factors that often trigger delirium in humans.

A third approach is based on the idea that the underlying cellular and molecular neurobiology and pathology may indeed be shared by mice. This includes activation of certain immune cells,[61] or the disruption of the blood-brain barrier integrity should be accompanied by behavioral features resembling human delirium.[62] However, we again

emphasize that key clinical features of delirium are both nonspecific and difficult to quantify, so many behavioral features associated with these interventions may be mistaken for those of delirium if factors confounding the tests are not carefully controlled for.

A fourth, and possibly more realistic, goal of the preclinical model is to interrogate specific biological mechanisms rather than recreate delirium in toto. Though this approach has been utilized for AD/ADRD with the use of transgenic APP models, it is important to note that these mice do not have AD/ADRD per se. As such, efforts to make them more directly and translationally relevant have included multigenic models and aged knock-in models. Preclinical models for delirium face a higher bar and greater challenges than for preclinical models of AD/ADRD given an absence of (a) a familial, hereditary-based component and (b) objective, validated neurobiology or pathology of delirium to guide potential mechanistic investigations.

A fifth approach relies on findings that some molecular and cellular responses to inflammatory or metabolic insults seem to be conserved across species,[63] suggesting an opportunity for validation in vitro and with empirical evidence from human biomarker studies. The cognitive effects of these insults in rodents does not need to precisely mimic delirium in humans to provide useful information. In other words, mechanisms hypothesized to contribute to delirium in humans may produce phenotypes in rodents that approximate features of human delirium. With this approach, the face validity of these phenotypes is improved if shown that the phenotypes in rodents are consistent with that of human delirium (eg, being transient, affecting attention, and higher prevalence or greater severity in older animals and in animals with underlying neurodegeneration).

Lastly, although current murine delirium models have illustrated moderate utility, improvement within the models requires further cross validation between clinical and preclinical research. For example, leveraging clinical specimens and established biomarkers from well-phenotyped human cohorts can be combined with preclinical models to further clarify the role of specific biomarkers involved in delirium.[64] In addition, clinical studies may identify genetic predispositions to inform model creation and distinguish among mechanistic subtypes or mechanism-phenotype associations. When revealed, effective pharmacological treatments for delirium in humans or treatments that disrupt the delirium-neurodegeneration link present powerful opportunities for validating models that may ultimately have clinical benefit. Work in animal models more closely related to humans (*eg*, in nonhuman primates [NHP]), may provide enhanced opportunities to obtain mechanistic data on critical pathways and targets, as opposed to studies conducted in more phylogenetically distant organisms (*eg*, mice). We acknowledge that capturing complex, human behaviors is more realistic in larger mammals, in particular in NHP; however, logistic or cost constraints may limit such studies.

1.5 | Phenotypic and behavioral assessments of delirium features in animal models

Human delirium is a clinical diagnosis that relies on a spectrum of observable behaviors and their timing. Many of these behaviors are compared against what the observer considers to be normal behavior. Similarly, a deviation in typical behavior, such as an acute change in “normal” behavior in rodents, for example, may be an indication of delirium, although

such deviations in behaviors may not necessarily mimic delirium in humans. As a starting point, one could use the Confusion Assessment Method (CAM) criteria[65] (widely applied in human delirium), for which specific domains can be tested in rodents. Noting the important DSM-5 criterion that the observed behavior "is not better explained by a preexisting, established, or evolving neurocognitive disorder," it is important to verify that observed mouse behavioral features are not assigned to delirium or to cognitive impairment if they are better explained by altered locomotor activity, reduced motivation, increased stress, or other aspects of sickness behavior.[66] Table 4 lists example behavioral tests (further discussed below) for rodents.

1.5.1 | Key delirium features

Acute onset and fluctuating course.: Acute onset and fluctuating course is a central criterion for the diagnosis of delirium. This is often difficult to assess in animals. The "acute" refers to the proximity of the behavioral change to a precipitant (eg, surgery or drug) and, to a lesser extent, the suddenness of the change. Frequent repeat testing is difficult because careful behavioral measurements can take hours and learning effects should be considered. Thus, the ease with which we can detect behavioral changes that are "acute" is diminished. However, changes that genuinely represent an acute onset change from baseline measures on the relevant parameters, such as working memory and attention, are possible to detect and have been performed.[44] Similarly, a "fluctuating course" requires repetitive evaluations with variable outcomes. This requires tracking individual animals rather than analyzing groups of animals (as is often done in animal research). Such approaches to detecting a fluctuating course have been reported.[35] While challenging to detect, demonstrating acute and fluctuating course is a key feature of delirium and reflects an important objective for the field.

Inattention.: "Inattentiveness" is another central criterion in the diagnosis of delirium in humans. Some rodent behavioral assays are reported to capture attention. This includes the five-choice serial reaction time task (5-CSRTT),[43] attentional set shifting,[40] and buried food test.[36,37] More recent work has stressed the importance of including distracting stimuli so that the animal must remain vigilant for the correct target stimuli. These "continuous performance tasks" have been reverse translated to humans and been shown to capture attentional deficit disorder[68] and therefore should be useful to assess attention or vigilance in animals. Although some of these tasks might be more specific for attention, in reality, the majority of rodent behavioral assays require a degree of attentiveness, as well as executive function, memory, and sensory abilities (eg, sight, sound, and smell). Even in nonmotivational tests (eg, novel object recognition and open field), the rodent must be attentive to its environment to display behavior that can be scored. Although attention is the only minimum required attribute, the rodent must also recognize the environment as novel or dangerous, which implies that memory and cognition are additional key attributes important for the detection of inattention. Similarly, tests that involve a reward (usually food) probe memory, sensory, and reward pathways that are incrementally more complex behaviors than isolated attention.[34] Thus, although a single animal assay for attention remains elusive and is unlikely to appropriately capture inattention, several overlapping tests that require attention may reveal a common defect (ie, change in attention). For example, NHP are

capable of several attentional tasks that mirror attention in humans (eg, visual search tasks). In such animal models, it is critical to rule out motivational, appetitive, locomotor, memory consolidation deficit, and severe sickness behavior before concluding that a given test demonstrates inattention.

Disorganized thinking.: In humans, disorganization of thought is determined on the basis of a set of established human norms to establish how an organized train of thought should progress. Although disorganized thinking in an animal might be inferred from its behavior (eg, not seeking food, exploring a familiar rather than a novel object, not being fearful in a known dangerous environment), violation of normative behaviors (eg, grooming a lower-ranking member or stealing food from a higher-ranking member of the troop, as can be observed in NHP) is difficult to objectively measure. This is further compounded by the knowledge that these measures are subject to important confounding factors. As with detecting the presence of inattention (described earlier), it is important that potential confounding factors are assessed to avoid mistaking changes in motivation, anxiety, or memory for disorganized thinking. Some questions to consider include the following: Is the animal motivated to seek or consume food? Is the animal's lack of fear explained by the failure to consolidate the memory of the fearful event?

Apart from NHP work, the field could additionally benefit from studies involving rodent models subject to assessments of cognitive disorganization (eg, prepulse inhibition, as used in schizophrenia[69] and traumatic brain injury[70] models). Though overt psychosis is more challenging to assess in rodents, newer computational-behavioral techniques may be useful for quantifying hallucination-like perception in mice.[72]

Altered level of consciousness.: Since assessing level of consciousness relies on generally primitive responses to overt stimuli, this is among the least difficult features to assess in an animal model. The speed and completeness of the righting reflex, for example, might be a gauge of intermediate levels of consciousness that could be consistent with, but nonspecific for, delirium. By evaluating an animal's response to overt stimuli, such as being placed in an unfamiliar environment (eg, open field) or having to interface with an unfamiliar object (eg, novel object recognition), it may be possible to gauge alterations in the level of consciousness. In a similar way, alterations in the level of consciousness may be inferred by changes in established animal behavior norms, such as typical behaviors that can be observed with home cage monitoring (ie, spontaneous locomotor activity, grooming, socializing, and rearing).

Arousal and delirium.: Because fluctuations in the level of arousal above and below one's baseline are a prominent feature of human delirium, alterations in the brain's arousal systems have long been implicated in delirium pathogenesis. For example, functional magnetic resonance imaging (fMRI) studies have suggested disruption of the ascending reticular activating system in patients with delirium admitted to a medicine ward.[74,75] Increased noradrenaline release from the locus coeruleus has also been associated with heightened noradrenergic transmission in the prefrontal cortex and hippocampus during delirium hyperactive states and linked to inattention and cognitive dysfunction.[76–79] Additionally, since sleep deprivation powerfully alters arousal level and sleep disturbances

are common in hospitalized delirious patients, it will be critical to investigate how triggers such as perioperative sleep dysregulation, aging, and anesthetic/sedative drugs may destabilize wake-sleep transitions and contribute to disordered arousal. To date, many of these questions remain open. Substantial research efforts will be needed to resolve whether a direct causative role of sleep disturbances in delirium exists and the underlying wake-sleep circuitry dysfunction.

Emergence from anesthesia might be utilized as a model of delirium. While emergence in the healthy brain typically requires only minutes, prolonged emergence, common in older adults, may resemble delirium. For example, stage 2 general anesthesia is generally an unconscious, hyperactive state associated with light anesthesia that phenotypically resembles hyperactive delirium.[80] In animals, it would be possible to maintain low concentrations of anesthetics to prolong such states in order to study them for delirium-like behaviors and biomarkers. Further support for the use of anesthesia in delirium research is that, at the network level, both human and rodent brains undergo pronounced fluctuations in activity at a steady concentration of anesthetic,[81–83] which may closely mimic the fluctuating nature of delirium.

1.5.2 | Minor features

Memory impairment.: There are many tests for memory, both spatial and contextual, in the rodent. Although the tests are mostly limited to spatial and olfactory memory in rodents, impairments in memory would reasonably be expected to accompany delirium in an animal, though not be specific to it.

Psychomotor agitation or retardation.: Enhanced or diminished motor activity over that normally seen for the time of day or age and sex of the animal might reflect psychomotor changes relevant to delirium and may provide an assay distinguishing hyper- from hypoactive delirium. Sophisticated “smart cages,” in addition to more conventional video-tracking systems, are readily available to monitor spontaneous locomotor activity. In addition, these monitoring systems, which have been adopted for complex analysis of mouse behavior video recordings to examine the impact of therapeutics,[73] can measure more elaborate behaviors such as grooming and rearing to assess some aspects of self-care and anxiety, which might also be altered in delirious states. Reduced time spent in the center of an open field may also indicate thigmotaxis, considered a marker of psychomotor agitation. In contrast, avoidance of open arms of the elevated plus maze may suggest the presence of associated anxiety-like behavior (Table 4). It remains important to determine whether motivational, energetic, or stress responses are causal with respect to altered locomotor activity.

Altered sleep-wake cycle.: As mentioned earlier, automated activity monitors have been validated for tracking sleep-wake cycles in rodents. Dysregulated sleep might reasonably be expected to accompany confusional and agitated states and are very common features in delirious patients. The primary advantages here are that (1) sleep is not a learned behavior or a motivational behavior and (2) sleep does not require the physical presence of the

investigator. Taken together, these advantages suggest that altered sleep-wake cycles should be free of the usual confounding factors present in many behavioral assays.

Potential pitfalls.: Beyond the obvious challenges of creating a human condition in preclinical models, many nuanced factors must be taken into account when developing models for the study of delirium. The need to assess cognition acutely, paired with the intended and unintended consequences of commonly employed vulnerability and precipitating factors, must be recognized. Because aging and neurodegeneration models are often used as *vulnerability* conditions in the field, methodical evaluation of these animals should be performed prior to the use of even common behavioral paradigms. For example, such animals are known to develop sensory loss[72,76] and impaired mobility,[77] which may limit the utility of behavioral paradigms requiring consistent responsiveness to sensory cues (eg, prepulse inhibition and many operant conditioning paradigms) or intact mobility (eg, Morris Water Maze), respectively. The often pleiotropic effects of precipitating factors must be similarly appraised. For example, when utilizing LPS or live-bacterial models of sepsis, loss of appetite and food motivation caused by sickness behavior should be considered. This potential experimental pitfall has been directly addressed through the development of a novel “escape from shallow water” T-maze for studying delirium, which strategically employs shallow water as a non-life-threatening, aversive motivator in an otherwise classic T-wave alternation task testing working memory.[40]

Necessity of behavioral assessment battery.: The implicit assumption of mapping these human phenotypic criteria of delirium to an animal is that they represent a similar neurobiological state. The validity of such an assumption is unknown. The behavioral response to, for example, a significant neuroinflammatory stress might simply be hypoactivity or sickness behavior in both human and animal. But the additional neurobiological vulnerabilities that convert sickness behavior into delirium could be different in animals, or at least have different thresholds. A related consideration is the potential need for normalization based on the animal’s level of activity, which could vary depending on the disease model and confound the assessment of certain behavioral assays.

It is clear that no single animal behavioral assay directly assesses a state analogous to human delirium and, because the cause of particular animal behaviors can only be inferred, that the behaviors may not be specific to a delirium-like state. Many tests, however, probe components of delirium. Attention and memory, as examples, are required in most animal behavior assays. Spontaneous, natural behaviors, such as that in open fields, novel object recognition, and sleep quality, might be included to broaden the behavioral repertoire, and these changes may also offer some explanatory aids to the interpretation of other tasks performed. In addition, if appropriately controlled, motivational assays could get closer to attention and disorganized thinking. Thus, we recommend that investigators who aim to characterize a delirium-like outcome use a battery of tests, when possible, to assess different cognitive, sensory, reflex, arousal, and reward networks. We recognize, however, that many questions regarding the use of these tests remain: How do we integrate individual tests in a battery? How do we account for key factors such as timing and sequence? And how should

the data be aggregated and analyzed? These questions underscore a growing need for more advanced methodological approaches to move the field forward.

Finally, the utility of measuring several parameters in each experiment needs to be balanced against the advantage of performing repeat evaluations, at intervals as closely spaced as practical, in order to track acute onset and fluctuating courses. Consistent with the latter, statistical analysis should track individuals, rather than just groups, over time in order to assess whether any findings are acute and fluctuating at an individual level. Imperfect as these tests are, the testing of potential treatments would provide a pathway to the eventual refinement of the models.

Ultimately, it is likely that the identification of the appropriate behavioral battery can only be established by first replicating conditions that have been strongly associated with human delirium (eg, age, inflammation, and drugs) and then characterizing the behavioral outcome, rather than trying to map CAM onto an animal, in other words, prioritizing construct validity rather than face validity. Evaluation of altered consciousness and disorganized thinking requires expert clinical judgment in humans and can be difficult to assess in animals since they are nonverbal. Therefore, an alternative starting point is to take a behavioral neuroscience approach and use well-characterized tasks, which unambiguously interrogate specific cognitive domains, in order to determine which traits are impaired in models with good construct validity. This iterative process should ultimately be useful in identifying behavioral patterns most representative of human delirium. Because no specific drug has been shown to mitigate delirium, using pharmacology to validate the state, as has been successfully done in schizophrenia and depression, seems implausible at the current time. Because delirium animal models continue to evolve, the testing of potentially effective treatments may provide a pathway to the eventual refinement and validation of the models.

1.6 | Biomarkers of delirium

In human studies to date, many biomarkers temporally associated with delirium have been identified (Table 5), with varying degrees of replication or validation. These biomarkers are presented here since they may provide targets against which animal models may be validated. Iterative refinement in translational bedside-to-bench and bench-to-bedside studies will allow cross-validation of these biomarkers and allow hypothesis generation for delirium neurobiology or pathology. Thus, although the biomarkers in Table 5 must be referred to only as “potential,” they hold promise for cross-validation studies to move the field of delirium forward.

In our usage, “biomarker” is used broadly and includes not only biofluid analytes but also neurophysiological and neuroimaging modalities. Currently, several clinical studies have described a number of biomarkers associated with human delirium; however, their specificity for delirium is not well established. Additionally, whether the association between a biomarker and clinical phenotype represents causality remains unclear. Biochemical, imaging, and neurophysiological markers associated with human delirium and measured in animals with delirium-like behavioral changes will enhance both the face and construct validity of the animal models and will facilitate moving beyond understanding the associations between a given biomarker and delirium to identifying causal mechanisms

associated with delirium. Nevertheless, once animal models of delirium-like behavior have been validated, they could be effectively used to identify new biomarkers in animals and subsequently to probe their validity in delirious patients, with the ultimate goal of illuminating the neurobiology and pathology of human delirium.

Delirium biomarkers may be grouped into two broad categories: (1) risk or predisposing markers (ie, present before the onset of delirium, indicating substrates conferring vulnerability) and (2) disease markers (ie, present during delirium, which may point to the mechanisms that directly precipitate or sustain delirium). Additionally, a third family of biomarkers may also be considered: outcome markers that are present after delirium resolves and indicate longer-term injury. It is important to note that some biomarkers may simultaneously belong to more than one category. We emphasize here that there is currently no validated biomarker for delirium. In fact, it is likely that a composite of biomarkers will be needed to provide high predictability.

Emerging evidence suggests that biomarkers that could bring mechanistic insights into delirium include biofluid assays for inflammation and brain cellular stress/injury, neurophysiological, and neuroimaging markers (Table 5). Research is urgently needed to clarify (1) the underlying brain injury mechanisms that trigger or sustain delirium, (2) the dysfunctional neural circuitries that underlie the delirium phenotype, and (3) interactions of biomarkers with clinical risk factors to cause long-term injury. Although it is possible to directly sample the animal brain, this only provides a view of the pathophysiology at a single point in time and cannot be directly cross-validated to the human brain. An important advantage of the biomarkers that will be discussed in what follows is that they can be obtained repetitively, providing a longitudinal view of the pathophysiology in an individual animal and compared directly to humans.

1.6.1 | Biofluid biomarkers—Biofluid biomarkers can be divided into markers reflecting (1) vulnerability for delirium and (2) the consequences of the precipitant. In the category of vulnerability for delirium markers, this would include markers of neurodegeneration such as those associated with AD/ADRD and perhaps inflammatory mediators that are associated with a variety of smoldering neuroinflammatory disorders. These biomarkers are typically not found in WT animal models, but rodents can be genetically modified and these biomarkers subsequently measured in tissue and biofluids. The category of markers reflecting the consequences of the precipitant can be further divided into a series of inflammatory mediators (reviewed in Reference 19), such as interleukin-6 (IL-6), tumor necrosis factor-alpha (TNFα), and high mobility group box protein-1 (HMGB1), markers readily measured in animals (see Table 5 for additional details and citations). These mediators may also cause neuronal injury, reflected by more durable biomarkers, similar to those predisposing to delirium vulnerability. In fact, this might be why an episode of delirium increases the risk of future delirium episodes.

Validating whether these biomarkers are causally linked to delirium remains a challenge since in humans such linkages are associative at best. Although controversial, it is plausible that delirium may reflect neuronal injury rather than a transient imbalance in neurotransmitter release or bioenergetics across networks and brain regions. Many neuronal

injury biomarkers exist, including glial fibrillary acid protein (GFAP), tau (and its isoforms), neurofilament light chain (NfL), and glial-derived protein S100 beta (S100β). Of note, however, some neuronal injury biomarkers, such as NfL and tau, are also found in abundance peripherally. Thus, if the delirium precipitant is direct tissue injury (surgery, trauma) or results in indirect tissue injury via hypoperfusion (hypovolemia, sepsis), then these and other biomarkers may reflect the severity of peripheral injury and not necessarily injury to the brain. Therefore, the attempted association of these biomarkers with delirium would have only indirect validity. This has been demonstrated in humans, where plasma NfL but not cerebrospinal fluid (CSF) NfL increased significantly after orthopedic surgery.[84] Likewise, proinflammatory mediators will be produced in the blood in all individuals after infection, injury, or surgery, indicating that these markers cannot be considered reliable biomarkers for delirium. These issues are mitigated in animals since blood, CSF, and brain tissue can each be available for assay, and once association is established, causation can be probed with appropriate interventions.

New generations of biofluid biomarkers might be of value in delirium. For example, microRNAs (miRNAs) are small, noncoding RNAs that act as rapid-response regulatory factors and have been investigated as brain injury biomarkers in a variety of settings. In a study of rugby players, a panel of plasma miRNAs linked to inflammation and to vascular integrity predicted with 95% accuracy which players with head injury would develop a concussion.[85] Though the miRNA source cannot be confirmed, the association is intriguing and may be translatable to animals.

Another novel biofluid biomarker includes plasma exosomes. Exosome “cargo” can contain proteins, mRNA, miRNA, and transcription factors, which provide a snapshot of activity in the cells and brain regions extruding the exosomes. For this reason, exosomes are under investigation as biomarkers of, for example, AD.[87] Comparison of brain-derived exosomal cargos or miRNAs in the blood of patients with and without delirium might provide novel delirium biomarkers, as well as potential targets for study in animals.

1.6.2 | Neurophysiological markers

Electroencephalography (EEG): Accumulating human data support electrophysiological monitoring as a promising biomarker of delirium.[3,87,88] Electroencephalography (EEG) can be used as an onsite diagnostic tool, especially useful for hypoactive delirium or when communication is compromised in patients recovering from general anesthesia or sedated in the ICU. Given the high temporal resolution, continuous EEG could help define the acute onset and fluctuating course of delirium, one of the key clinical features in the CAM.[65] It is important to note that (1) the neurophysiological changes accompanying delirium could represent a common neurobiological consequence of multiple divergent pathologies of delirium,[87,89] as well as other neurophysiologic states, and (2) the exact relationship between EEG/sleep-wake disturbances and delirium remains unclear (ie, whether sleep-wake disturbances are a consequence of or a predisposing factor to delirium).

Because of the largely human nature of the delirium syndrome, it will be imperative to cross-validate EEG studies in animals with those in humans (and vice versa), when feasible. In this iterative framework, animal EEG studies that mirror EEG metrics of

delirium in humans will be helpful in the validation of those animal models. In turn, EEG studies in reliable animal models for delirium may help identify novel biomarkers that relate to specific mechanistic pathways and, thus, may be useful in extending human clinical findings. With this in mind, the following sections describe electrophysiological and imaging studies of human delirium that in our view can be conducted in animals and would be beneficial, though to-date have received little attention.

Diffuse slowing of the EEG has been described in delirious patients for decades[89–92] and has several advantages. Delirium is associated with an increase in delta (<4 Hz) and theta (4 to 8 Hz) osccillations and a decrease in alpha (>8 Hz) oscillations on the EEG.[93–100] These changes correlate with cognitive performance and delirium severity.[101] In fact, one study reported that quantitative EEG could distinguish delirious from nondelirious patients with a sensitivity of 100% and a specificity of 99%.[96] An additional advantage of EEG is that it provides measures of functional connectivity, which has also been shown to be impaired and less integrated in delirious patients following stressors of various types.[95,102,103] Lastly, a final advantage relates to the relative ease of measuring electrical activity in awake and unrestrained animals. Taken together, these advantages provide a strong rationale for the use of EEG in animal model validation.

Neurophysiological signatures similar to delirium also occur at loss and return of consciousness during anesthesia and recovery, and these could be explored in animals to shed light on the commonly observed altered level of consciousness in delirium.[81,104–106] However, caution is needed because different anesthetics induce distinct neurophysiological changes[81,104–106] and, thus, may not precisely mimic the neurophysiological correlates of altered levels of consciousness during delirium. In addition, these neurophysiological markers may differ depending on the etiology of delirium.[87,89] Nevertheless, the animal models and experimental paradigm that have been used to investigate anesthesia-induced altered levels of consciousness might prove to be of value if adapted to studies of delirium.

EEG features observed in delirium require further validation for (1) temporal correlation with behavioral features, (2) association with the presumed pathophysiological mechanisms of delirium, and (3) distinction from or agreement with EEG features of other neurocognitive disorders that share some behavioral aspects with delirium. Recently, LPS-induced inflammation was found to produce slow waves and decrease anteroposterior connectivity in the electroencephalogram, along with behavioral quiescence and elevated cortical cytokines in aged mice.[107] These data could then be considered consistent with hypoactive delirium and support the use of EEG markers to distinguish delirium types in animals. To establish EEG markers for all types of delirium including hyperactive and mixed type, it will be essential to demonstrate temporal correlations between these markers and delirium behaviors in the model. Although these behaviors can be challenging to interpret in rodents (see preceding discussion), human-like hallucinatory and locomotor behaviors are often present in NHP models.[108–110] Further, reliable cognitive task performance that enables testing of attention, altered levels of consciousness, and dis-orientation is possible in NHP,[104–106] and NHP have demonstrated that recordings of EEG across the neocortex is comparable to those seen in humans.[111,112]

Evoked potentials: Additionally, evoked potentials may be used as a neurophysiological marker for delirium. A recent prospective cohort study applied somatosensory (SSEP) and brainstem auditory (BAEP) evoked potentials in deeply sedated critically ill patients.[113] The results suggest that early impairment of the intrapontine conduction time is associated with postsedation delirium, pointing to the possible impairment of the ascending reticular activation system in the pathogenesis of delirium. SSEP and BAEP can be a useful complementary tool for delirium in high-risk patients, but they have yet to be tested in animal models.

Invasive electrophysiology: Direct, more invasive neurophysiological activities, such as local field potentials, single neuron activity, and brain microdialysis (directly measuring neurotransmitter levels), could provide critical insights into the neurophysiological mechanisms of delirium. While becoming possible in human cohorts (eg, electrocorticography recordings prior to epilepsy resection and brain-computer interface studies[114–118]), direct neural recordings are primarily applicable only to preclinical animal models. In particular, the brain regions that represent specific cognitive functions or neurotransmitter systems can be directly tested in animal models.[87,89] Note that, to date, direct neurophysiological recordings from animal models of delirium remain very limited.[119]

1.6.3 | Imaging markers—Imaging modalities offer two main advantages in the study of delirium pathophysiology in animal models. First, imaging can serve as a bridge between delirium in human subjects, NHP, and smaller animal species. Behavioral data and imaging markers from humans and NHP could guide studies in smaller animals (eg, rodents with mechanistic, tissue-level confirmation that cannot be performed in humans or NHP). Another important advantage of imaging is that certain brain regions that have been associated with delirium cannot be readily assessed through specific behavioral, electrophysiological, or biochemical tests but can be evaluated via imaging. For example, the posterior cingulate cortex, a component of the default mode network that has been implicated in human delirium, is readily visualized with positron emission tomography (PET).

Functional magnetic resonance imaging: Human fMRI studies suggest disruption in the central executive network (active at external task demands) and the default mode network (active at wakeful rest) in patients with delirium. Both networks involve the prefrontal cortex[74,114] and are consistent with the network dysconnectivity hypothesis of delirium.[87,89] These region-specific hypotheses for delirium could be tested in animal models with direct neurophysiological recording; however, unlike EEG, the low temporal resolution of most types of brain imaging require that the animal be motionless for long periods, implying the use of anesthetics, which could confound the results.

Positron emission tomography: A number of imaging modalities exist with the potential to shed light on both anatomic and circuit changes in delirium. However, practical considerations have limited the use of many of these modalities and, thus, have constrained validation of associated imaging biomarkers. PET uses a shorter imaging paradigm, and

most of its tracers are minimally affected by a brief sedation since some interrogate processes that are not being modulated rapidly (eg, microglial activation). Another compelling aspect of PET imaging is the ability to identify and create novel probes for the process, receptor, or other desired target. A number of PET tracers, either for clinical research use or for clinical use in humans, are in the late stages of development or nearing approval and are able to detect neuroinflammation, white matter tract injury, apoptosis, autophagy, and changes in metabolism in human subjects with delirium. These tracers include sphingosine-1-phosphate receptor 1, purinoceptor 7, and peripheral-type benzodiazepine receptor 28 for microglial activation, alongside reactive tracers that indicate innate immune activation in the brain (M1, ROS-Trace). These tracers may be able to provide powerful information regarding inflammation, stress, and neuronal/microglia injury processes that supports or complements delirium-like behavioral changes and other neurophysiological markers of delirium in the same animal model.

In summary, the aforementioned biomarkers are highlighted as ones having significant potential to allow cross-validation of neurobiology and pathology between humans and animals. This would not only enable greater face validity of behavioral assays but would also provide a window into the underlying mechanisms of delirium.

1.7 | Recommendations for preclinical and translational models for delirium

1.7.1 | General—General guidelines for animal experimental design were recently published (Planning Research and Experimental Procedures on Animals: Recommendations for Excellence [PREPARE])[119] and are relevant to preclinical delirium research. As of this writing, an animal model that recapitulates human delirium with high fidelity has not been reported, so it is important for the investigator to clearly specify what feature of human delirium is being studied and the underlying premise. How is this feature in the rodent (or other animal model) similar to that in human delirium, and is the underlying physiology, where known, similar? In what ways is it different, and does this challenge the relevance to delirium? While it will remain unclear whether such a reductionistic approach (ie, feature by feature) is valid for a complex syndrome like delirium, it nevertheless recognizes the imperfect translation of human delirium to rodents (among other animal models). The following guidelines and suggestions are not intended to be proscriptive but rather represent what we consider to be best practices to cautiously advance this type of preclinical research. Given that there is much room for growth in the field, our guidelines and recommendations reflect what is lacking in the literature, and we anticipate that future, more specific guidelines, building on PREPARE,[119] will be adopted once the field of animal models for delirium has undergone further advances.

1.7.2 | Investigator expertise—Animal behavior can be as varied as that of humans and be surprisingly responsive to environmental cues. Thus, it is imperative that investigators be exceptionally consistent in their handling of animals and in their administration of behavioral tasks. This can extend, for example, from the time of day to the identity and gender of the investigator, and even to the clothes they wear.[120,121] Thus, many investigators are now turning to animal behavior core labs to administer tests and collect data in order to enhance reproducibility.

1.7.3 | Species, strain, and sex—The choice of animal species always reflects a balance between cost, regulation, and relevance. Rodents (specifically mice) probably represent the optimal balance, but even here, it can be expensive to maintain colonies to an appropriately vulnerable age. Some mouse behavioral paradigms are validated and standardized, and the relatively low cost allows relatively large sample sizes. Furthermore, when pathophysiological hypotheses for delirium can be formulated, the relative ease of validation based on genetic manipulation is a significant strength of the murine model. Delirium occurs in both male and female humans, so both sexes should be studied in animal studies.

Mice are available in a bewildering variety of largely inbred strains (eg, C57BL/6). Inbred strains offer the advantage of genetic similarity, enabling the elimination of genetic diversity as a confounder in experimental studies. Genetic drift reduces this advantage after about 20 generations, so these inbred strains require "refreshing." Genetic drift also occurs in transgenic or knock-out (knock-in) animals, potentially reducing or eliminating the hoped-for vulnerability factors. Although genetic similarity is an advantage to the experimentalist in one sense, it is a disadvantage when considering that the human we are trying to model is enormously diverse. It should be recalled that not all older adults enter a state of delirium, likely reflecting genetic and other sources of diversity. Outbred mice, such as the diversity outbred (DO) mouse,[122] are preferred in this case, but the investigator should be prepared for greater variability in the results, which suggests that greater numbers of animals are needed. Finally, in general, mice are exceptionally resilient to all manner of insults, though it could be expected that outbred strains and WT animals would be more so.

1.7.4 | Vulnerability—Young, healthy humans, like young, healthy WT animals, rarely enter a state of delirium (*ie*, are not considered vulnerable). We therefore consider it essential to introduce a vulnerability factor into the model to enhance construct validity. As summarized earlier and in Table 6, this can be advanced age (we suggest 18–24 months), genetic changes (eg, introduction of human AD/ADRD-associated genes), drugs or toxins, polypharmacy, or some combination. We also advocate that the experimental design include control groups of animals without the vulnerability factor to demonstrate its independent role, especially when evaluating underlying pathophysiology.

1.7.5 | Precipitant—Because of the acute nature of most human delirium, it is widely assumed that some precipitant superimposed on the given vulnerability is required to trigger a state of delirium. Reproducing the multiple factors that precipitate delirium in a clinical setting complicates the study design (and feasibility), especially when working with older and frail animals. Nevertheless, one or more such factors could be objectively introduced into the experimental design (eg, surgery and/or anesthesia, infection, injection of inflammatory mediators, and environmental alteration, such as noise, movement, lights, socialization, sleep disruption, drugs, or pain). The resulting behavioral phenotype should be monitored closely and repeatedly at intervals designed to capture the "fluctuating" nature of the behavioral change. Some precipitants may be so stressful or disruptive (eg, sepsis) that a state of delirium could be entered without the need for a vulnerability. Also, since some precipitants may be physiologically disruptive (eg, anesthesia or LPS injection), we consider

it important to monitor and report physiology (eg, temperature, heart and ventilation rate, oxygen saturation, and blood pressure) to the extent possible in the animal. While it remains contentious in humans, some animal studies have indicated that some general anesthetics alone can cause subsequent acute cognitive disturbances, as well as neurodegenerative features.[37,87] However, it is critical to note that general anesthesia in a rodent is not analogous to that in a human patient. Patients undergoing anesthesia have their physiology carefully and continually monitored while rodents are only superficially monitored, if at all. In fact, in some rodent studies, prolonged, multidrug, repeated anesthetic exposures can cause sufficient physiological disturbance (eg, hypoxemia, hypotension, or hypothermia) to independently cause cognitive disturbances.

1.7.6 | Outcome measures and validation—Two types of outcome measures should be considered in the experimental design. First, the behavioral phenotype is crucial, as it is (currently) the primary means of alignment with the human condition. As discussed earlier, human delirium involves several behavioral domains (from awareness to motor), but what is measured does not necessarily need to encompass every domain, depending on the goals of the experiment. In fact, a thorough behavioral evaluation in the rodent may be counterproductive, in that it requires so much time that an ability to assess the acute and fluctuating nature of changes is lost. This balance between temporal issues and behavioral thoroughness should be decided ahead of time and aligned with the delirium feature chosen for study.

The second outcome measure would include that reflecting pathophysiology. As discussed earlier and presented in Table 5, this would include various biofluid biomarkers, tissue analysis (eg, enzyme-linked immunosorbent assays, histochemistry, and morphology), electrophysiology, or imaging. These outcomes are critically important as they reflect the primary advantage of animal models: to align the relationship between pathophysiology and behavior in animals, in the hope of translating knowledge to humans. The temporal relationship between behavior and pathophysiology is similarly important, so, to the extent possible, we suggest contemporaneous measurement of behavior and pathophysiology. Secondary outcomes should also leverage knowledge for other disciplines that can help to validate the findings from animal models. For example, microphysiological organ-on-chip technologies can be applied to deconstruct a neuroimmune interaction of relevance to delirium pathogenesis and treatment with human cells.[123] Standardized chips and devices can be used with various cell sources,[124] including human induced pluripotent stem cells (hiPSCs), to define new targets and high-throughput drug discovery applications. Ongoing advances in behavioral assays with the use of artificial intelligence, automated tracking, and machine learning provide novel ways to evaluate complex behaviors, enhancing rigor and reproducibly, and remain largely underutilized in this field.

1.7.7 | Statistics—Behavioral measures have a degree of subjectivity and, therefore, have a larger coefficient of variation than biofluid biomarkers, for example. Because of this, we suggest that the investigative team incorporate the largest number of animals they can afford or handle, recognizing the costs associated with increasing numbers of animals. Acknowledging that variability and effect size estimates are key metrics in determining the

number of animals per group to be studied, we cautiously suggest inclusion of ≥20 animals/group given that effect sizes are rarely known at the time of study inception but are generally small (10–20%). Therefore, behavioral studies with <20 animals per group are likely to be underpowered, whereas this might not be the case with more objective continuous measures like biofluid biomarkers. Ideally, and where possible, a power analysis should be conducted based on the effect size in preliminary data or the literature. When such effect size estimates are unknown, a clinically meaningful effect size could be considered. Analyses should be focused on both individuals and groups rather than only on groups to assess key delirium features like acute onset and fluctuating course.

1.7.8 | Reporting—Reproducibility and rigor require a priori study design (including a statistical approach), reliable reporting of all details, and depositing of data in publicly available databases after publication (eg, Harvard Dataverse, AlzPed). Access to repositories (including the NIA Biobank and other resources referenced on the NIDUS website < https://deliriumnetwork.org/ >) offer unapparelled opportunities for data mining and for developing bioinformatic resources as encouraged by funding agencies. PREPARE reporting guidelines[119] should be followed.

1.8 | Conclusions and future directions

It is not reasonable to expect any animal model to mimic human delirium with high fidelity. Thus, our overarching recommendation is to utilize animal models to advance our understanding for specific, key features and key pathological hallmarks of delirium as progressively informed by the clinical literature. Animal models may be useful for bidirectional translation of associative studies in humans, followed by more mechanistic studies in animals, and then returning to the human for attempts at mechanistic intervention. As more knowledge about delirium accumulates, animal models should become an integral part of a systems approach to investigating delirium neurobiology, one that interfaces with strategies such as computer neuroscience, machine learning, and artificial intelligence, with the common goal of improving the prevention, diagnosis, and treatment of this complex clinical syndrome. Attention to construct validity (vulnerability and precipitant) as well as face validity (behavior) in the animal is critical to lend credence to the associated pathophysiology. Future work needs to carefully assess and optimize animal physiology during the precipitant phase and to incorporate multiple behavioral paradigms that include a temporal component and analyze the data as individuals rather than groups. Multidisciplinary approaches will likely provide unique opportunities to study delirium in preclinical models. In this regard, NIDUS facilitates the creation of large-scale multidisciplinary projects centered on modeling delirium in animals. The expectation for this and, hopefully, more consortium-based work is that several questions will be tackled in parallel by multidisciplinary teams, overall enhancing rigor and reproducibly for the various protocols and offer designs that better align with clinical randomized controlled trials, as opposed to a single laboratory observation. Delirium, as both an isolated condition and a strong risk factor for AD/ADRD, will continue to impact our society, as has been repeatedly demonstrated by the rapid increase in our aging population and the aftermath of COVID-19. In this regard, it is of paramount importance to advance preclinical research in this field

to better understand how predisposing and precipitating factors cause delirium and which interventions will be able to prevent and treat this common neurological complication.

ACKNOWLEDGMENTS

The authors thank the scientists work was funded primarily by a grant from the National Institute on Aging (R33AG071744) to NIDUS (SKI). Other support was provided by funding from the National Institute on Aging (K01AG057836 [SMV]), National Heart, Lung, and Blood Institute (K08HL159353 [JAH]), and the Alzheimer's Association (AARF-18-560786, AARG-22-917342 [SMV]). This manuscript was facilitated by the Alzheimer's Association International Society to Advance Alzheimer's Research and Treatment (ISTAART) through the Perioperative Cognition and Delirium professional interest area (PIA). The views and opinions expressed herein by the authors represent those of the authors and do not necessarily reflect those of the PIA membership, ISTAART, or the Alzheimer's Association.

Funding information

National Institute on Aging, Grant/Award Numbers: R33AG071744, K01AG057836; Alzheimer's Association, Grant/Award Numbers: AARF-18-560786, AARG-22-917342; National Heart, Lung, and Blood Institute, Grant/Award Number: K08HL159353

REFERENCES

1. Breitbart W, Rosenfeld B, Roth A, et al. The memorial delirium assessment scale. J Pain Symptom Manage. 1997;13(3):128–137. [PubMed: 9114631]
2. Cole MG. Delirium in elderly patients. Am J Geriatr Psychiatry. 2004;12(1):7–21. [PubMed: 14729554]
3. Wilson JE, Mart MF, Cunningham C, et al. Delirium. Nat Rev Dis Primers. 2020;6(1):90. [PubMed: 33184265]
4. Goldberg TE, Chen C, Wang Y, et al. Association of Delirium with long-term cognitive decline: a meta-analysis. JAMA Neurol. 2020;77(11):1373. [PubMed: 32658246]
5. Leslie DL, Inouye SK. The importance of delirium: economic and societal costs. J Am Geriatr Soc. 2011;59:S241–S243. [PubMed: 22091567]
6. Vasilevski EE, Chandrasekhar R, Holtze CH, et al. The cost of ICU delirium and coma in the Intensive Care Unit patient. Med Care. 2018;56(10):890–897. [PubMed: 30179988]
7. Oh E, Fong TG, Hshieh TT, Inouye SK. Delirium in older persons: advances in diagnosis and treatment (systematic review). JAMA. 2017;318:1161–1174. [PubMed: 28973626]
8. Gou RY, Hshieh TT, Marcantonio ER, et al. One-year medicare costs associated with delirium in a cohort of older patients undergoing major elective surgery. JAMA Surg. 2021;156:430–442.
9. Khachaturian A, Hayden KM, Devlin JM, et al. International drive to illuminate delirium: a developing public health blueprint for action. Alzheimers Dement. 2020;16(5):711–725. [PubMed: 32212231]
10. Inouye SK, Charpentier PA. Precipitating factors for delirium in hospitalized elderly persons. Predictive model and interrelationship with baseline vulnerability. JAMA. 1996;275:852–857. [PubMed: 8596223]
11. Sukoff Rizzo SJ, Masters A, Onos KD, et al. Improving preclinical to clinical translation in Alzheimer's disease research. Alzheimers Dement (NY). 2020;6(1):e12038.
12. Pang H, Kumar S, Ely EW, Gezalian MM, Lahiri S. Acute kidney injury-associated delirium: a review of clinical and pathophysiological mechanisms. Crit Care. 2022;26(1):258–270. [PubMed: 36030220]
13. Fong TG, Inouye SK. The inter-relationship between delirium and dementia: the importance of delirium prevention. Nat Rev Neurol. 2022. epub ahead of print.
14. Banerjee R, Rai a, Iyer SM, Narwal S, Tare M. Animal models in the study of Alzheimer's disease and Parkinson's disease: a historical perspective. Anim Models Exp Med. 2022;00:1–11.

15. Yednock TA, Cannon C, Fritz LC, Sanchez-Madrid F, Steinman L, Karin N. Prevention of experimental autoimmune encephalomyelitis by antibodies against $\alpha 4\beta 1$ integrin. Nature. 1992;356: 63–66. [PubMed: 1538783]
16. Houser SR, Margulies KB, Murphy AM, et al. Animal models of heart failure: a scientific statement from the American Heart Association. Am Heart Assoc Circ Res. 2012;111(1):131–150.
17. FDA. Aducanumab (marketed as Aduhelm) Information. Accessed on September 20, 2022 https://www.fda.gov/drugs/postmarket-drug-safety-information-patients-and-providers/aducanumab-marketed-aduhelm-information
18. Morrice JR, Gregory-Evans CY, Shaw CA. Animal models of amyotrophic lateral sclerosis: a comparison of model validity. Neural Regen Res. 2018;13(12):2050–2054. [PubMed: 30323119]
19. Yang T, Velagapudi R, Terrando N. Neuroinflammation after surgery: from mechanisms to therapeutic targets. Nat Immunol. 2020;21(11):1319–1326. [PubMed: 33077953]
20. Manabe T, Heneka MT. Cerebral dysfunctions caused by sepsis during ageing. Nat Rev Immunol. 2022;22(7):444–458. [PubMed: 34764472]
21. Lopez MG, Hughes CG, DeMatteo A, et al. Intraoperative oxidative damage and delirium after cardiac surgery. Anesthesiology. 2020;132:551–561. [PubMed: 31770146]
22. Fong TG, Vasunilashorn SM, Ngo L, et al. Association of plasma neurofilament light with postoperative delirium. Ann Neurol. 2020;88(5):984–994. [PubMed: 32881052]
23. Field RH, Gossen A, Cunningham C. Prior pathology in the basal forebrain cholinergic system predisposes to inflammation-induced working memory deficits: reconciling inflammatory and cholinergic hypotheses of delirium. J Neurosci. 2012;31(18):6288–6294.
24. Biomarkers Definitions Working Group. Biomarkers and surrogate endpoints: preferred definitions and conceptual framework. Clin Pharmacol Ther. 2001;69:89–95. [PubMed: 11240971]
25. Oh ES, Akeju O, Avidan MS, et al. A roadmap to advance delirium research: recommendations from the NIDUS scientific think tank. Alzheimer's Dement. 2020;16:726–733. [PubMed: 32291901]
26. Tinneke D, Thomas S, Maarten VR. Animal models in translational medicine: validation and prediction. New Horiz Transl Med. 2014;2:5–11.
27. Belzung C, Lemoine M. Criteria of validity for animal models of psychiatric disorders: focus on anxiety disorders and depression. Biol Mood Anxiety Disord. 2011;1:9. [PubMed: 22738250]
28. Inouye SK, Studenski S, Tinetti ME, Kuchel GA. Geriatric syndromes: clinical, research, and policy implications of a core geriatric concept. J Am Geriatr Soc. 2007;55:780–791. [PubMed: 17493201]
29. Gill TM, Williams CS, Tinetti ME. The combined effects of baseline vulnerability and acute hospital events on the development of functional dependence among community-living older persons. J Gerontol A Biol Sci Med Sci. 1999;54:377–383.
30. Pessoa L The entangled brain. J Cognitive Neurosci. 2022;15:1–2.
31. Senatorov VV Jr, Friedman AR, Milikovsky DZ, et al. Blood-brain barrier dysfunction in aging induces hyperactivation of TGFbeta signaling and chronic yet reversible neural dysfunction. Sci Transl Med. 2019;11:eaaw8283.
32. Castanon-Cervantes O, Wu M, Ehlen JC, et al. Dysregulation of inflammatory responses by chronic circadian disruption. J Immunol. 2010;185:5796–5805. [PubMed: 20944004]
33. Culley DJ, Snayd M, Baxter MG, et al. Systemic inflammation impairs attention and cognitive flexibility but not associative learning in aged rats: possible implications for delirium. Front Aging Neurosci. 2014;6:107. [PubMed: 24959140]
34. Godbout JP, Chen J, Abraham J, et al. Exaggerated neuroinflammation and sickness behavior in aged mice following activation of the peripheral innate immune system. FASEB J. 2005;19:1329–1331. [PubMed: 15919760]
35. Illendula M, Osuru HP, Ferrarese B, et al. Surgery, anesthesia and intensive care environment induce delirium-like behaviors and impairment of synaptic function-related gene expression in aged mice. Front Aging Neurosci. 2020;12:542421. [PubMed: 33088271]
36. Liufu N, Liu L, Shen S, et al. Anesthesia and surgery induce age-dependent changes in behaviors and microbiota. Aging. 2020;12:1965–1986. [PubMed: 31974315]

37. Yang S, Gu C, Mandeville ET, et al. Anesthesia and surgery impair blood-brain barrier and cognitive function in mice. Front Immunol. 2017;8:902. [PubMed: 28848542]
38. Zhou Y, Wang J, Li X, et al. Neuroprotectin D1 protects against postoperative delirium-like behavior in aged mice. Front Aging Neurosci. 2020;12:582674. [PubMed: 33250764]
39. Davis DHJ, Skelly DT, Murray C, et al. Worsening cognitive impairment and neurodegenerative pathology progressively increase risk for delirium. Am J Geriatr Psychiatry. 2015;23:403–415. [PubMed: 25239680]
40. Murray C, Sanderson DJ, Barkus C, et al. Systemic inflammation induces acute working memory deficits in the primed brain: relevance for delirium. Neurobiol Aging. 2012;33:603–616. [PubMed: 20471138]
41. Kealy J, Murray C, Griffin EW, et al. Acute inflammation alters brain energy metabolism in mice and humans: role in suppressed spontaneous activity, impaired cognition, and delirium. J Neurosci. 2020;40:5681–5696. [PubMed: 32513828]
42. Skelly DT, Griffin ÉW, Murray CL, et al. Acute transient cognitive dysfunction and acute brain injury induced by systemic inflammation occur by dissociable IL-1-dependent mechanisms. Mol Psychiatry. 2019;24:1533–1548. [PubMed: 29875474]
43. Wang P, Velagapudi R, Kong C, et al. Neurovascular and immune mechanisms that regulate postoperative delirium superimposed on dementia. Alzheimers Dement. 2020;16(5):734–749. [PubMed: 32291962]
44. Velagapudi R, Subramaniyan S, Xiong C, et al. Orthopedic surgery triggers attention deficits in a delirium-like mouse model. Front Immunol. 2019;10:2675. [PubMed: 31911786]
45. Yang T, Xu G, Newton PT, et al. Maresin 1 attenuates neuroinflammation in a mouse model of perioperative neurocognitive disorders. Br J Anaesth. 2019;122:350–360. [PubMed: 30770053]
46. Peng M, Zhang C, Dong Y, et al. Battery of behavioral tests in mice to study postoperative delirium. Sci Rep. 2016;6:29874. [PubMed: 27435513]
47. Ren Q, Peng M, Dong Y, et al. Surgery plus anesthesia induces loss of attention in mice. Front Cell Neurosci. 2015;9:346. [PubMed: 26441522]
48. Andonegui G, Zelinski EL, Schubert CL, et al. Targeting inflammatory monocytes in sepsis-associated encephalopathy and long-term cognitive impairment. JCI Insight. 2018;3(9):e99364. [PubMed: 29720578]
49. Zhang Z, Ma Q, Shah B, et al. Neuroprotective effects of annexin A1 tripeptide after deep hypothermic circulatory arrest in rats. Front Immunol. 2017;8:1050. [PubMed: 28912778]
50. Fonken LK, Weil ZM, Nelson RJ. Mice exposed to dim light at night exaggerate inflammatory responses to lipopolysaccharide. Brain Behav Immun. 2013;34:159–163. [PubMed: 24012645]
51. Semmler A, Hermann S, Mormann F, et al. Sepsis causes neuroinflammation and concomitant decrease of cerebral metabolism. J Neuroinflammation. 2008;5:38. [PubMed: 18793399]
52. Rosengarten B, Hecht M, Auch D, et al. Microcirculatory dysfunction in the brain precedes changes in evoked potentials in endotoxin-induced sepsis syndrome in rats. Cerebrovasc Dis. 2007;23: 140–147. [PubMed: 17124395]
53. Huizer-Pajkos A, Kane AE, Howlett SE, et al. Adverse geriatric outcomes secondary to polypharmacy in a mouse model: the influence of aging. J Gerontol A Biol Sci Med Sci. 2016;71:571–577. [PubMed: 25940962]
54. Mach J, Gemikonakli G, Logan C, et al. Chronic polypharmacy with increasing Drug Burden Index (DBI) exacerbates frailty and impairs physical function, with effects attenuated by deprescribing, in aged mice. J Gerontol A Biol Sci Med Sci. 2021;76(6):1010–1018. [PubMed: 32147704]
55. Kincheski GC, Valentim IS, Clarke JR, et al. Chronic sleep restriction promotes brain inflammation and synapse loss, and potentiates memory impairment induced by amyloid-beta oligomers in mice. Brain Behav Immun. 2017;64: 140–151. [PubMed: 28412140]
56. Tieges Z, Evans JJ, Neufeld K, MacLullich AMJ. The neuropsychology of delirium: advancing the science of delirium. Int J Geriatr Psychiatry. 2017;33(11):1501–1511. [PubMed: 28393426]
57. Beckers J, Wurst W, de Angelis MH. Towards better mouse models: enhanced genotypes, systemic phenotyping and envirotype modelling. Nat Rev Genet. 2009;10:371–380. [PubMed: 19434078]

58. Goldberg IJ, Sharma G, Fisher EA. Atherosclerosis: making a U turn. Annu Rev Med. 2020;71:191–201. [PubMed: 31986087]
59. Scearce-Levie K, Sanchez PE, Lewcock JW. Leveraging preclinical models for the development of Alzheimer disease therapeutics. Nat Rev Drug Discov. 2020;19:447–462. [PubMed: 32612262]
60. Kleinert M, Clemmensen C, Hofmann SM, et al. Animal models of obesity and diabetes mellitus. Nat Rev Endocrinol. 2018;14:140–162. [PubMed: 29348476]
61. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. J Immunol. 2004;172:2731–2738. [PubMed: 14978070]
62. O'Brown NM, Pfau SJ, Gu C. Bridging barriers: a comparative look at the blood-brain barrier across organisms. Genes Dev. 2018;32:466–478. [PubMed: 29692355]
63. Longo VD, Finch CE. Evolutionary medicine: from dwarf model systems to healthy centenarians? Science. 2003;299(5611):1342–1346. [PubMed: 12610293]
64. David-Bercholz J, Acker A, Caceres AI, et al. Conserved YKL-40 changes in mice and humans after postoperative delirium. Brain Behav Immun Health. 2022;26:100555. [PubMed: 36457825]
65. Inouye SK, van Dyck CH, Alessi CA, Balkin S, Siegal AP, Horwitz RI. Clarifying confusion: the confusion assessment method. A new method for detection of delirium. Ann Intern Med. 1990;113:941–948. [PubMed: 2240918]
66. Cunningham C, Sanderson DJ. Malaise in the water maze: untangling the effects of LPS in IL-1B on learning and memory. Brain Behav Immun. 2008;22(8):1117–1127. [PubMed: 18640811]
67. Carr ZJ, Miller L, Ruiz-Velasco V, Kunselman AR, Karamchandani K. In a model of neuroinflammation designed to mimic delirium, quetiapine reduces cortisol secretion and preserves reversal learning in the attentional set shifting task. J Neuroimm Pharmacol. 2019;14(3):383–390.
68. Roberts BV, Young JW. Translational cognitive systems: focus on attention. Emerg Top Life Sci. 2022;6(5):529–539. [PubMed: 36408755]
69. Brody SA, Dulawa SC, Conquet F, Geyer MA. Assessment of a pre-pulse inhibition deficit in mutant mouse lacking mGlu5 receptors. Mol Psychiatry. 2004;9(1):35–41. [PubMed: 14699440]
70. Browne CA, Wulf HA, Jacobson ML, Oyola MG, Wu TJ, Lucki I. Long-term increase in sensitivity to ketamine's behavioral effects in mice exposed to mild blast induced traumatic brain injury. Exp Neurol. 2022;350:113963. [PubMed: 34968423]
71. Schmack K, Bosc M, Ott T, Sturgill JF, Kepecs A. Striatal dopamine mediates hallucination-like perception in mice. Science. 2021;372(6537):eabf4740.
72. Shuboni-Mulligan DD, Young DL Jr, De La Crus Minyety J, et al. Impact of age of the circadian visual system and the sleep-wake cycle in mus musculus. NPJ Aging Mech Dis. 2021;7:10. [PubMed: 33947857]
73. Khroyan TV, Zhang J, Yang L, et al. Rodent motor and neuropsychological behavior measured in home cages using the integrated modular platform SmartCageTM. Clin Exp Pharmacol Physiol. 2012;39(7):614–622. [PubMed: 22540540]
74. Choi SH, Lee H, Chung TS, et al. Neural network functional connectivity during and after an episode of delirium. Am J Psychiatry. 2012;169:498–507. [PubMed: 22549209]
75. Boukrina O, Kowalczyk M, Koush Y, et al. Brain network dysfunction in poststroke delirium and spatial neglect: an fMRI study. Stroke. 2022;53(3):930–938. [PubMed: 34619987]
76. Buscher N, van Dorsselaer P, Steckler T, Talpos JC. Evaluating aged mice in three touchscreen tests that differ in visual demands: impaired cognitive function and impaired visual abilities. Behav Brain Res. 2017;333:142–149. [PubMed: 28690184]
77. Justice JN, Carter CS, Beck HH, et al. Battery of behavioral tests in mice that models age-associated changes in human motor function. Age. 2014;36(2):583–592. [PubMed: 24122289]
78. Hansen N, Rediske AI. The locus coeruleus noradrenaline system in delirium. Fron Aging Neurosci. 2021;13:784356.
79. Plini ERG, O' Hanlon E, Boyle R, et al. Examining the role of the noradrenergic locus coeruleus for predicting attention and brain maintenance in healthy old age and disease: an MRI structural study for the Alzheimer's disease neuroimaging initiative. Cells. 2021;10(7):1829–1858. [PubMed: 34359997]

80. Laycock JD. Signs and stages of anaesthesia. A restatement. Aneaesthesia. 1953;8(1):15–20.
81. Patel SR, Ballesteros JJ, Ahmed OJ, et al. Dynamics of recovery from anaesthesia-induced unconsciousness across primate neocortex. Brain. 2020;143:833–843. [PubMed: 32049333]
82. Hudson AE, Calderon DP, Pfaff DW, Proekt A. Recovery of consciousness is mediated by a network of discrete metastable activity states. Proc Natl Acad Sci. 2014;111(25):9283–9288. [PubMed: 24927558]
83. Acker L, Ha C, Zhou J, et al. Electroencephalogram-based complexitiy measures as predictors of postoperative neurocognitive dysfunction. Front Syst Neurosci. 2021;15:71879.
84. Halaas NB, Blennow K, Idland AV, et al. Neurofilament light in serum and cerebrospinal fluid of hip fracture patients with delirium. Dement Geriatr Cogn Disord. 2018;46(5–6):346–357. [PubMed: 30522125]
85. Di Pietro V, O'Halloran P, Watson CM, et al. Unique diagnostic sigantures of concussion in the saliva of male athletes: the Study of Concussion in Rugby Union through microRNAs (SCRUM). Br J Sports Med. 2021;55:1395–1404. [PubMed: 33757972]
86. Goetzl EJ. Advancing medicine for Alzheimer's disease: a plasma neural exosome platform. FASEB J. 2020;34(10):13079–13084. [PubMed: 32856798]
87. van der Kooi AW, Leijten FS, van der Wekken RJ, Slooter AJ. What are the opportunities for EEG-based monitoring of delirium in the ICU? J Neuropsychiatry Clin Neurosci. 2012;24:472–477. [PubMed: 23224454]
88. Eckenhoff RG, Maze M, Xie Z, et al. Perioperative neurocognitive disorder: state of the preclinical science. Anesthesiology. 2020;132:55–68. [PubMed: 31834869]
89. Maldonado JR. Delirium pathophysiology: an updated hypothesis of the etiology of acute brain failure. Int J Geriatr Psychiatry. 2018;33:1428–1457. [PubMed: 29278283]
90. Brenner RP. Utility of EEG in delirium: past views and current practice. Int Psychogeriatr. 1991;3:211–229. [PubMed: 1811775]
91. Jacobson S, Jerrier H. EEG in delirium. Semin Clin Neuropsychiatry. 2000;5:86–92. [PubMed: 10837097]
92. Zisook S, Braff DL. Delirium: recognition and management in the older patient. Geriatrics. 1986;41(6):67–70. 72–73,77–80.
93. Kimchi EY, Neelagiri A, Whitt W, et al. Clinical EEG slowing correlates with delirium severity and predicts poor clinical outcomes. Neurology. 2019;93:e1260–e1271. [PubMed: 31467255]
94. Palanca BJA, Wildes TS, Ju YS, Ching S, Avidan MS. Electroencephalography and delirium in the postoperative period. Br J Anaesth. 2017;119:294–307. [PubMed: 28854540]
95. Tanabe S, Mohanty R, Lindroth H, et al. Cohort study into the neural correlates of postoperative delirium: the role of connectivity and slow-wave activity. Br J Anaesth. 2020;125:55–66. [PubMed: 32499013]
96. Fleischmann R, Tränkner S, Bathe-Peters R, et al. Diagnostic performance and utility of quantitative EEG analyses in delirium: confirmatory results from a large retrospective case-control study. Clin EEG Neurosci. 2019;50:111–120. [PubMed: 29631447]
97. Numan T, van den Boogaard M, Kamper AM, Rood PJT, Peelen LM, Slooter AJC. Delirium detection using relative delta power based on 1-minute single-channel EEG: a multicentre study. Br J Anaesth. 2019;122:60–68. [PubMed: 30579407]
98. van der Kooi AW, Zaal IJ, Klijn FA, et al. Delirium detection using EEG: what and how to measure. Chest. 2015;147:94–101. [PubMed: 25166725]
99. Evans JL, Nadler JW, Preud'homme XA, et al. Pilot prospective study of post-surgery sleep and EEG predictors of post-operative delirium. Clin Neurophysiol. 2017;128:1421–1425. [PubMed: 28618293]
100. Nielsen RM, Urdanibia-Centelles O, Vedel-Larsen E, et al. Continuous EEG monitoring in a consecutive patient cohort with sepsis and delirium. Neurocrit Care. 2020;32:121–130. [PubMed: 30891696]
101. Thomas C, Hestermann U, Kopitz J, et al. Serum anticholinergic activity and cerebral cholinergic dysfunction: an EEG study in frail elderly with and without delirium. BMC Neurosci. 2008;9:86. [PubMed: 18793418]

102. Numan T, Slooter AJC, van der Kooi AW, et al. Functional connectivity and network analysis during hypoactive delirium and recovery from anesthesia. Clin Neurophysiol. 2017;128:914–924. [PubMed: 28402867]
103. van Montfort SJT, van Dellen E, Stam CJ, et al. Brain network disintegration as a final common pathway for delirium: a systematic review and qualitative meta-analysis. Neuroimage Clin. 2019;23: 101809. [PubMed: 30981940]
104. Ballesteros JJ, Briscoe JB, Ishizawa Y. Neural signatures of α2-Adrenergic agonist-induced unconsciousness and awakening by antagonist. Elife. 2020;9:e57670. [PubMed: 32857037]
105. Ballesteros JJ, Huang P, Patel SR, Eskandar EN, Ishizawa Y. Dynamics of ketamine-induced loss and return of consciousness across primate neocortex. Anesthesiology. 2020;132:750–762. [PubMed: 32053559]
106. Ishizawa Y, Ahmed OJ, Patel SR, et al. Dynamics of propofol-induced loss of consciousness across primate neocortex. J Neurosci. 2016;36:7718–7726. [PubMed: 27445148]
107. Sultan ZW, Jaeckel ER, Krause BM, et al. Electrophysiological signatures of acute systemic lipopolysaccharide-induced inflammation: potential implications for delirium science. Br J Anaesth. 2021;126:996–1008. [PubMed: 33648701]
108. Castner SA, Goldman-Rakic PS. Amphetamine sensitization of hallucinatory-like behaviors is dependent on prefrontal cortex in nonhuman primates. Biol Psychiatry. 2003;54:105–110. [PubMed: 12873799]
109. Brewster JM, Siegel RK, Johnson CA, Jarvik ME. Observational determination of dose-response curves in hallucinogen-treated monkeys. Int Pharmacopsychiatry. 1976;11:102–108. [PubMed: 820666]
110. Schlemmer RF Jr, Davis JM. A primate model for the study of hallucinogens. Pharmacol Biochem Behav. 1986;24:381–392. [PubMed: 3952128]
111. Sandhaeger F, von Nicolai C, Miller EK, Siegel M. Monkey EEG links neuronal color and motion information across species and scales. Elife. 2019;8:e45645. [PubMed: 31287792]
112. Teichert T Tonal frequency affects amplitude but not topography of rhesus monkey cranial EEG components. Hear Res. 2016;336: 29–43. [PubMed: 27085798]
113. Zauner C, Gendo A, Kramer L, et al. Impaired subcortical and cortical sensory evoked potential pathways in septic patients. Crit Care Med. 2002;30:1136–1139. [PubMed: 12006815]
114. Kramer DR, Barbaro MF, Lee M, et al. Electrocorticographic changes in field potentials following natural somatosensory percepts in humans. Exp Brain Res. 2019;237:1155–1167. [PubMed: 30796470]
115. Jian T, Pellizzer G, Asman P, et al. Power modulations of ECoG alpha/beta gamma bands correlate with time-derivative of force during hand grasp. Front Neurosci. 2020;14:100. [PubMed: 32116533]
116. Pels EGM, Aarnoutse EJ, Leinders S, et al. Stability of a chronic implanted brain-computer interface in late-stage amyotrophic lateral sclerosis. Clin Neurophysiol. 2019;130(10):1798–1803. [PubMed: 31401488]
117. Leinders S, Vansteensel MJ, Branco MP, et al. Dorsolateral prefrontal cortex-based control with an implanted brain-computer interface. Sci Rep. 2020;10:5448. [PubMed: 32214120]
118. Mamad O, Islam MN, Cunningham C, Tsanov M. Differential response of hippocampal and prefrontal oscillations to systemic LPS application. Brain Res. 2018;1681:64–74. [PubMed: 29294350]
119. Smith AJ, Clutton RE, Lilley E, Hansen KEA, Brattelid T. PREPARE: guidelines for planning animal research and testing. Lab Anim. 2018;52(2):135–141. [PubMed: 28771074]
120. Chapman CD, Benedict C, Schiöth HB. Experimenter gender and replicability in science. Sci Adv. 2018;4(1):e1701427. [PubMed: 29349293]
121. Georgiou P, Zanos P, Mou TCM, et al. Experimenters' sex modulates mouse behaviors and neural responses to ketamine via corticotropin releasing factor. Nat Neurosci. 2022;25(9):1191–1200. [PubMed: 36042309]
122. Neuner SM, Heuer SE, Huentelman MJ, O'Connell KMS, Kaczorowski CC. Harnessing genetic complexity to enhance translatability of Alzheimer's disease mouse models: a path toward precision medicine. Neuron. 2019;101(3):399–411. [PubMed: 30595332]

123. Yang T, Velagapudi R, Kong C, et al. Protective effects of omega-3 fatty acids in a blood-brain barrier-on-chip model and on postoperative delirium-like behaviour in mice. Br J Anaesth. 2022. epub ahead of print.

124. McCloskey MC, Kasap P, Ahmad SD, et al. The modular μSiM: a mass produced, rapidly assesmbled, and reconfigurable platform for the study of barrier tissue models in vitro. Adv Healthc Mater. 2022;11(18):e2200804. [PubMed: 35899801]

125. Rashid MH, et al. Interleukin-6 mediates delirium-like phenotypes in a murine model of urinary tract infection. J Neuroinflamm. 2021;18(1):247.

126. Trzepacz PT, Leavitt M, Ciongoli K. An animal model for delirium. Psychosomat. 1992;33(4):404–415.

127. Cunningham C, Campion S, Lunnon K, et al. Systemic inflammation induces acute behavioral and cognitive changes and accelerates neurodegenerative disease. Biol Psychiatry. 2009;65(4):304–312. [PubMed: 18801476]

128. Hovens IB, van Leeuwen BL, Nyakas C, Heineman E, van der Zee EA, Schoemaker RG. Postoperative cognitive dysfunction and microglial activation in associated brain regions in old rats. Neurobiol Learn Mem. 2015;118:74–79. [PubMed: 25460037]

129. Kong F-J, Ma L-L, Zhang H-H, Zhou J-Q. Alpha 7 nicotinic acetylcholine receptor agonist GTS-21 mitigates isoflurane-induced cognitive impairment in aged rats. J Surg Res. 2015;194:255–261. [PubMed: 25450597]

130. Li Z, Cao Y, Li L, et al. Prophylactic angiotensin type 1 receptor antagonism confers neuroprotection in an aged rat model of postoperative cognitive dysfunction. Biochem Biophys Res Comm. 2014;449(1):74–80. [PubMed: 24814703]

131. Wang Y, He H, Li D, et al. The role of the TLR4 signaling pathway in cognitive deficits following surgery in aged rats. Mol Med Rep. 2013;7(4):1137–1142. [PubMed: 23426570]

132. Zhao L, Wang F, Gui B, Hua F, Qian Y. Prophylactic lithium alleviates postoperative cognition impairment by phosphorylating hippocampal glycogen synthase kinase-3β (Ser9) in aged rats. Exp Gerontol. 2011;46(12):1031–1036. [PubMed: 21945291]

133. Lopez-Rodriguez AB, Hennessy E, Murray CL, et al. Acute systemic inflammation exacerbates neuroinflammation in Alzheimer's disease: IL-1β drives amplified responses in primed astrocytes and neuronal network dysfunction. Alzheimers Dement. 2021;17(10):1735–1755. [PubMed: 34080771]

134. McGarry N, Murray C, Garvey S, et al. Double stranded RNA drives anti-viral innate immune responses, sickness behavior and cognitive dysfunction dependent on dsRNA lengthen, IFNAR1 expression and age. Brain Behav Immun. 2021;95:413–428. [PubMed: 33892139]

135. Braff DL, Swerdlow NR, Geyer MA. Symptom correlates of prepulse inhibition deficits in male schizophrenic patients. Am J Psychiatry. 1999;156:596–602. [PubMed: 10200740]

136. Cadenhead KS, Serper Y, Braff DL. Transient versus sustained visual channels in the visual backward masking deficits of schizophrenia patients. Biol Psychiatry. 1998;43(2):132–138. [PubMed: 9474445]

137. Wesson DW, Borkowski AH, Landreth GE, Nixon RA, Levy E, Wilson DA. Sensory network dysfunction, behavioral impairments, and their reversibility in an Alzheimer's B-Amyloidosis mouse model. J Neurosci. 2011;31(44):15962–15971. [PubMed: 22049439]

138. Filali M, Lalonde R, Rivest S. Anomalies in social behaviors and exploratory activities in an APPswe/PS1 mouse model of Alzheimer's disease. Physiol Behav. 2011;104(5):880–885. [PubMed: 21640739]

139. Huang Y, Li L, Gan Y, et al. Sedentary behaviors and risk of depression: a meta-analysis of prospective studies. Transl Psychiatry. 2020;10(1):26. [PubMed: 32066686]

140. Marin C, Vilas D, Langdon C, et al. Olfactory dysfunction in neurodegenerative diseases. Curr Allergy Asthma Rep. 2018;18(8):42. [PubMed: 29904888]

141. Antunes M, Biala G. The novel object recognition memory: neurobiology, test procedure, and its modifications. Cogn Process. 2012;13(2):93–110. [PubMed: 22160349]

142. Leger M, Quiedeville A, Bouet V, et al. Object recognition test in mice. Nat Protoc. 2013;8(12):2531–2537. [PubMed: 24263092]

143. Bains RS, Wells S, Sillito RR, et al. Assessing mouse behavior throughout the light/dark cycle using automated in-cage analysis tools. J Neurosci Methods. 2018;300:37–47. [PubMed: 28456660]

144. Fisher SP, Godinho SIH, Pothecary CA, et al. Rapid assessment of sleep-wake behavior in mice. J Biol Rhythms. 2012;27(1):48–58. [PubMed: 22306973]

145. Poljak A, Hill M, MacLullich AM, et al. Quantitative proteomics of delirium cerebrospinal fluid. Transl Psychaitry. 2014;4(11):e477.

146. Vasunilashorn SM, Dillon ST, Chan NY, et al. Proteome-wide analysis using SOMAscan identifies and validates chitinase-3-like protein 1 as a risk and disease marker of delirium among older adults undergoing major elective surgery. J Gerontol A Biol Sci Med Sci. 2022;77(30):484–493. [PubMed: 35239952]

147. Westhoff D, Witlox J, van Aalst C, et al. Preoperative protein profiles in cerebrospinal fluid in elderly hip fracture patients at risk for delirium: a proteomics and validation study. BBA Clin. 2015;28:1155–1122.

148. Menzenbach J, Frede ST, Petras J, et al. Perioperative vascular biomarker profiling in elective surgery patients developing postoperative delirium: a prospective cohort study. Biomedicines. 2021;9(5):553. [PubMed: 34063403]

149. Forget M-F, Del Degtan S, Leblanc J, et al. Delirium and inflammation in older adults hospitalized for COVID-19: a cohort study. Clin Interv Aging. 2021;16:1223–1230. [PubMed: 34234422]

150. Khan SH, Lindroth H, Jawed Y, et al. Serum biomarkers in postoperative delirium after esophagectomy. Ann Thorac Surg. 2022;113(3):1000–1007. [PubMed: 33774004]

151. Knaak C, Vorderwulbecke G, Spies C, et al. C-reactive protein for risk prediction of post-operative delirium and post-operative neurocognitive disorder. Acta Anaesthesiol Scand. 2019;63(10):1282–1289. [PubMed: 31283835]

152. McKay TB, Rhee J, Colon J, et al. Preliminary study of serum biomarkers associated with delirium after major cardiac surgery. J Cardiothorac Vasc Anesth. 2022;36(1):118–124. [PubMed: 34112563]

153. Klimiec-Moskal E, Pasinska P, Kowalska K, et al. Elevated plasma levels of galectin-3 binding protein are associated with post-stroke delirium – A pilot study. J Neuroimmunol. 2021;356:577579. [PubMed: 33901789]

154. Adamis D, van Goold W, Eikelenboom P. Consistent patterns in the inconsistent associations of insulin-like growth factor 1 (IGF-1), C-Reactive Protein (C-RP) and interleukin 6 (IL-6) levels with delirium in surgical populations. A systematic review and meta-analysis. Arch Gerontol Geriatr. 2021;97:104518. [PubMed: 34536657]

155. Brattinga B, Plas M, Spikman JM, et al. The association between the inflammatory response following surgery and post-operative delirium in older oncological patients: a prospective cohort study. Age Ageing. 2022;51(2):237.

156. Chan CK, Song Y, Greene R, et al. Meta-analysis of ICU delirium biomarkers and their alignment with the NIA-AA Research Framework. Am J Crit Care. 2021;30(4):312–319. [PubMed: 34195769]

157. Zheng GZ, Chen XF, Chen LW, Luo ZR. Thyroid hormone, cortisol, interleukin-2, and procalcitonin regulate postoperative delirium in acute type A aortic dissection patients. BMC Cardiovasc Disord. 2022;22(1):503. [PubMed: 36434500]

158. Rhee J, Kuznetsov A, McKay T, et al. Serum proteomics of older patients undergoing major cardiac surgery: identification of biomarkers associated with postoperative delirium. Front Aging Neurosci. 2021;13:699763. [PubMed: 34456709]

159. Lu X-C, Lin Y, Wu Q-S, et al. Plasma interleukin-6 is a potential predictive biomarker for postoperative delirium among acute type A aortic dissection patients treated with open surgical repair. J Cardiothorac Surg. 2021;16(1):146. [PubMed: 34044881]

160. Conti E, Andreoni S, Tomaselli D, et al. Serum DBI and biomarkers of neuroinflammation in Alzheimer's disease and delirium. Neurol Sci. 2021;42(3):1003–1007. [PubMed: 32705487]

161. McNeil JB, hughes CG, Girard T, et al. Plasma biomarkers of inflammation, coagulation, and brain injury as predictors of delirium duration in older hospitalized patients. PLoS One. 2019;14(12):e0226412. [PubMed: 31856187]
162. Khan BA, Perkins AJ, Prasad NK, et al. Biomarkers of delirium duration and delirium severity in the ICU. Crit Care Med. 2020;48(3):353–361. [PubMed: 31770149]
163. Neerland BE, Hall RJ, Seljeflot I, et al. Associations between delirium and preoperative cerebrospinal fluid C-reactive protein, interleukin-6, and interleukin-6 receptor in individuals with acute hip fracture. J Am Geriatr Soc. 2016;64(7):1456–1463. [PubMed: 27341529]
164. Kowalska K, Klimiec E, Weglarczyk K, et al. Reduced ex vivo release of proinflammatory cytokines and elevated plasma interleukin-6 are inflammatory signatures of post-stroke delirium. J Neuroinflamm. 2018;15(1):111.
165. Kazmierski J, Miler P, Pawlak A, et al. Elevated monocyte chemoattractant protein-1 as the independent risk factor of delirium after cardiac surgery. A prospective cohort study. J Clin Med. 2021;10(8):1587. [PubMed: 33918634]
166. Hatta K, Kishi Y, Takeuchi T, et al. The predictive value of a change in natural killer cell activity for delirium. Prog Neuropsychopharmacol Biol Psychiatry. 2014;48:26–31. [PubMed: 24063857]
167. Menzenbach J, Frede S, Petras J, et al. Perioperative vascular biomarkers profiling in elective surgery patients developing postoperative delirium: a prospective cohort study. Biomedicines. 2021;9(5):553. [PubMed: 34063403]
168. Lin X, Tang J, Liu C, et al. Cerebrospinal fluid cholinergic biomarkers are associated with postoperative delirium in elderly patients undergoing total hip/knee replacement: a prospective cohort study. BMC Anesthesiol. 2020;20(1):246. [PubMed: 32988381]
169. Forget M-F, Del Degan S, Leblanc J, et al. Delirium and inflammation in older adults hospitalized for COVID-19: a cohort study. Clin Interv Aging. 2021;16:1223–1230. [PubMed: 34234422]
170. Fong TG, Vasunilashorn SM, Gou Y, et al. Association of CSF Alzheimer's disease biomarkers with postoperative delirium in older adults. Alzheimers Dement (N Y). 2021;7(1):e12125. [PubMed: 33748398]
171. Szwed K, Szwed M, Kozakiewicz M, et al. Circulating microRNAs and novel proteins as potential biomarkers of neurological complications after heart bypass surgery. J Clin Med. 2021;10(14):3091. [PubMed: 34300256]
172. Cooper J, Stukas S, Hoiland RL, et al. Quantification of neurological blood-based biomarkers in critically ill patients with coronavirus disease 2019. Crit Care Explor. 2020;2(10):e0238. [PubMed: 33063038]
173. Gailiusas M, Adrejaitiene J, Sirvinskas E, et al. Association between serum biomarkers and postoperative delirium after cardiac surgery. Acta Med Litu. 2019;26(1):8–10. [PubMed: 31281210]
174. Wanderlind ML, Goncalves R, Tomasi CD, Dal-Pizzol F, Ritter C. Association of neurogranin with delirium among critically ill patients. Biomark Med. 2020;14(17):1613–1617. [PubMed: 33336596]
175. Reznik ME, Kalgara R, Moodly S, et al. Common biomarkers of physiologic stress and associations with delirium in patients with intracerebral hemorrhage. J Crit Care. 2021;64:62–67. [PubMed: 33794468]
176. Ida M, Takeshita Y, Kawaguchi M. Preoperative serum biomarkers in the prediction of postoperative delirium following abdominal surgery. Geriatr Gerontol Int. 2020;20(12):1208–1212. [PubMed: 33084189]
177. Mietani K, Hasegawa-Moriyama M, Inoue R, et al. Elevated neuron-specific enolase level is associated with postoperative delirium and detection of phosphorylated neurofilament heavy subunit: a prospective observational study. PLoS One. 2021;16(11): e0259217. [PubMed: 34797829]
178. Gao Y, Duan J, Ji H, Lu W. Levels of S100 calcium binding protein B (S100B), neuron-specific enolase (NSE), and cyclophilin A (CypA) in the serum of patients with severe craniocerebral injury and multiple injuries combined with delirium transferred from the ICU and their prognostic value. Ann Palliat Med. 2021;10(3):3371–3378. [PubMed: 33849122]

179. Casey CP, Lindroth H, Mohanty R, et al. Postoperative delirium is associated with increased plasma neurofilament light. Brain. 2020;143(1):47–54. [PubMed: 31802104]
180. Parker M, White M, Casey C, et al. Cohort analysis of the association of delirium severity with cerebrospinal fluid amyloid-tauneurodegeneration pathologies. J Gerontol A Biol Sci Med Sci. 2021. epub ahead of print.
181. Zhang X, Lyu Y, Wang D. S100β as a potential biomarker of incident delirium: a systematic review and meta-analysis. Minerva Anestesiol. 2020;86(8):853–860. [PubMed: 32449338]
182. Ballweg T, White M, Parker M, et al. Association between plasma tau and postoperative delirium incidence and severity: a prospective observational study. Br J Anaesth. 2021;126(2):458–466. [PubMed: 33228978]
183. Hayhurst CJ, Patel MB, McNeil JB, et al. Association of neuronal repair biomarkers with delirium among survivors of critical illness. J Crit Care. 2020;56:94–99. [PubMed: 31896448]
184. Ma JR, Fan MM, Wang ZS. Age, preoperative higher serum cortisol levels, and lower serum acetylcholine levels predict delirium after percutaneous coronary intervention in acute coronary syndrome patients accompanied with renal dysfunction. Indian J Psychiatry. 2020;62(2):172–177. [PubMed: 32382177]
185. Adam EH, Haas V, Lindau S, Zacharowski K, Scheller B. Cholinesterase alterations in delirium after cardiosurgery: a German monocentric prospective study. BMJ Open. 2020;10(1):e031212.
186. Michels B, Holzamer A, Graf BM, et al. Butyrylcholinesterase as a perioperative complication marker in patients after transcatheter aortic valve implantation: a prospective observational study. BMJ Open. 2021;11(7):e042857.
187. Henjum K, Godang K, Quist-Paulsen E, et al. Cerebrospinal fluid catecholamines in delirium and dementia. Brain Commun. 2021;3(3):fcab121.
188. Han Y, Chen W, Song Y, et al. Proteomic analysis of preoperative CSF reveals risk biomarkers of postoperative delirium. Front Psychiatry. 2020;11:170. [PubMed: 32194463]
189. Koponen H, Partanen J, Pääkkönen A, Mattila E, Riekkinen PJ. EEG spectral analysis in delirium. J Neurol Neurosurg Psychiatry. 1989;52:980–985. [PubMed: 2795067]
190. Mikola A, Sarkela MO, Walsh TS, Lipping T. Power spectrum and cross power spectral density based EEG correlates of intensive care delirium. Annu Int Conf IEEE Eng Med Biol Soc. 2019;2019:4562–4565. [PubMed: 31946880]
191. van Dellen E, van der Kooi AW, Numan T, et al. Decreased functional connectivity and disturbed directionality of information flow in the electroencephalography of intensive care unit patients with delirium after cardiac surgery. Anesthesiology. 2014;121:328–335. [PubMed: 24901239]
192. Fleischmann R, Traenkner S, Kraft A, Schmidt S, Schreiber SJ, Brandt SA. Delirium is associated with frequency band specific dysconnectivity in intrinsic connectivity networks: preliminary evidence from a large retrospective pilot case-control study. Pilot Feasibility Stud. 2019;5:2. [PubMed: 30631448]
193. Brown CH, Faigle R, Klinker L, et al. The association of brain MRI characteristics and postoperative delirium in cardiac surgery patients. Clin Ther. 2015;37(12):2686–2699. [PubMed: 26621626]
194. Omiya H, Yoshitani K, Yamada N, et al. Preoperative brain magnetic resonance imaging and postoperative delirium after off-pump coronary artery bypass grafting: a prospective cohort study. Can J Anesth. 2015;62(6):595–602. [PubMed: 25652160]
195. Shioiri A, Kurumaji A, Takeuchi T, et al. White matter abnormalities as a risk factor for postoperative delirium revealed by diffusion tensor imaging. Am J Geriatr Psychiatry. 2010;18(8):743–753. [PubMed: 20220599]
196. Otomo S, Maekawa K, Goto T, et al. Pre-existing cerebral infarcts as a risk factor for delirium after coronary artery bypass graft surgery. Intercat Cardiovasc Thorac Surg. 2013;17(5):799–804.
197. Root JC, Pryor KO, Downey R, et al. Association of pre-operative brain pathology with post-operative delirium in a cohort of non-small cell lung cancer patients undergoing surgical resection. Psychooncology. 2013;22(9):2087–2094. [PubMed: 23457028]
198. Morandi A, Rogers BP, Gunther ML, et al. The relationship between delirium duration, white matter integrity, and cognitive impairment in Intensive Care Unit survivors as determined by diffusion tensor imaging. Crit Care Med. 2012;40(7):2182–2189. [PubMed: 22584766]

199. Gunther ML, Morandi A, Krauskopf E. The association between brain volumes, delirium duration and cognitive outcomes in intensive care unit survivors: the VISIONS cohort magnetic resonance imaging study. Crit Care Med. 2012;40(7):2022–2032. [PubMed: 22710202]

200. Haggstrom LR, Nelson JA, Wegner EA, Caplan GA. 2-F-fluoro-2-deoxyglucose positron emission tomography in delirium. J Cereb Blood Flow Metab. 2017;37(11):3556–3567. [PubMed: 28350285]

201. Fong TG, Bogardus ST, Daftary A, Auerbach E, et al. Cerebral perfusion changes in older deliriuos patients using 99mTc HMPAO SPECT. J Gerontol A Biol Sci Med Sci. 2006;61(12):1294–1299. [PubMed: 17234823]

RESEARCH IN CONTEXT

1. **Systematic review**: The authors reviewed the literature on animal models for delirium using traditional sources (eg, PubMed). Although human studies of delirium have advanced our understanding of delirium epidemiology and intervention strategies, our knowledge of delirium neurobiology remains limited. Animal models for delirium could advance our knowledge of delirium neurobiology and help develop new prevention and treatment approaches.
2. **Integration**: Although the application of animal models of delirium remains in a nascent stage, there is a need for rigorous, high-quality science in this area of research to advance understanding of its complex neurobiology.
3. **Future directions**: This article discusses the application of animal models for delirium, focusing on (a) challenges and strategies for replicating elements of human delirium in animals; (b) utility of fluid, neurophysiology, and neuroimaging markers in animal models; and (c) recommendations for best practices for the development and validation of animal models for delirium.

TABLE 1

Uses of animal models in delirium research

Conceptual goal	Practical examples of experimental questions
• Advance pathophysiological and mechanistic understanding of delirium – Apply experimental manipulations to test mechanistic hypotheses – Determine causal mechanisms and pathways – Develop, test, and refine specific interventions for prevention and treatment	• Does exercise preconditioning reduce incidence/ severity of delirium in animal models and through which mechanistic pathways?
• Validate and extend clinical findings – Directly link mechanisms to pathophysiological, phenotypic, or neuroanatomic abnormalities	• Is slow EEG activity causally associated with inflammation in an animal model ofdelirium? • Is inflammation-related EEG slowing mechanistically associated with a phenotype of behavioral quiescence?
• Serve as a platform for validation of human biomarkers of delirium	• Do proteins identified from proteomic approaches in human samples associate with rodent behavioral tests align with human delirium features?

Abbreviation: EEG, electroencephalogram.

TABLE 2

Criteria for assessing validity of animal models

Validity type	Criteria for assessment
Face validity	Model should mimic aspects of the clinical presentation (see Table 4 for specific examples of behavioral tests relevant to delirium in rodents)
Construct validity	Model should produce signs of the condition by mimicking the types of etiological situations that lead to this symptomology
Predictive validity	Model should respond to specific treatments in a manner similar to patients that experience the disorder
Target validity	Model should demonstrate impact on specific pathophysiological pathways known to be associated with the condition

TABLE 3

Overview of select animal models for delirium

Model description (animal)	Reference	Age	Vulnerability factor	Precipitant	Model details/effects
Female C57BL/6 Mice	[125]	6–7 months	None	Urinary tract infection	Frontal and hippocampal functional impairments, specifically increased thigmotaxis in open field, and reduced spontaneous alternations in Y-maze correlating with neuronal injury marker, cleaved caspase-3
C57BL/6-ME7 Mice	[40]	8–12 weeks	1 microliter of 10% w/v scrapie (ME7 strain)-infected C57BL6 brain homogenate	100 μg/kg of LPS IP	Extracellular amyloidosis, synaptic loss, robust neurodegeneration, and progressive cognitive decline; diminished performance on T-maze test performed at 12 weeks post-disease initiation
C57BL/6 - hypocholinergic model Mice	[23]	Not stated	Preexisting neurodegeneration of basal forebrain cholinergic system	100 μg/kg LPS	Depletion of cholinergic neurons in basal forebrain and decreased innervation of hippocampus; diminished performance on T-maze test performed at 1 month post-cholinergic lesioning
Male Wistar Rats	[126]	Adult	None	IV administration of anticholinergic drug atropine	Impaired attention and memory, sleep-wake cycle reversal, and impaired focus, increased irritability, and fluctuating levels of activity
C57BL/6-ME7 Mice	[127]	Not stated	One μl of a 10% w/v scrapie (ME7 strain)-infected C57BL/6 brain homogenate	100 μg/kg of LPS IP	Y-maze performed 12 weeks post-disease initiation. Diminished performance on Y-maze test, motor tasks, and suppressed locomotor test; acutely impaired motor coordination and muscle strength, earlier onset of disease-associated impairments
Wistar rats	[128]	25 months	Age	Clamping upper mesenteric artery under sevoflurane anesthesia	Memory and learning impairment after surgery
Sprague-Dawley rats	[129]	20months	Age	Anesthesia only 4 h isoflurane 1.3%	Memory and learning impairment
Sprague-Dawley rats	[130]	20months	Age	Laparotomy under isoflurane anesthesia	Memory and learning impairment, diminished performance on Morris water maze test
Sprague-Dawley rats	[131]	22–23 months	Age	Splenectomy under isoflurane anesthesia	Memory and learning impairments, diminished performance on Morris water maze reversal test
Sprague-Dawley rats	[132]	18 months	Age	Laparotomy	Impaired spatial memory and diminished performance on Morris water maze (latency+ distance)
Fischer 344 rats	[33]	24 months	Age	50 μg/kg of LPS IP	Impaired reversal learning and attentional shifts without affecting discrimination learning in attentional set-shifting task, indicating deficit in attention and cognitive flexibility
Male BALB/cmice	[34]	20–24 months	Age	0.33 mg/kg of LPS	Neurobehavioral impairments consistent with delirium, depression, and early onset of neurodegeneration
Female C57BL/6J mice	[37]	18 months	Age	Anesthesia, laparotomy	Cognitive impairment was measured using Morris water maze, and Barnes maze
Female C57BL/6 mice	[38]	18 months	Age	Laparotomy under isoflurane anesthesia	Time-dependent impairment in buried food test, open field, and learned behaviors (Y-maze test) induced by surgery/anesthesia in aged mice

Model description (animal)	Reference	Age	Vulnerability factor	Precipitant	Model details/effects
Female C57BL/6J-ME7 mice	41	5–8 months	One microl of 10% w/v scrapie (ME7 strain)-infected C57BL6 brain homogenate	100 or 250 μg/kg of LPS IP	Impaired locomotor and cognitive function (open field test and escape-from-water paddling T-maze)
MaleC57BL/6 mice	44	12 weeks	Age	Tibial facture under isoflurane anesthesia	Five-choice serial reaction time task as a measure of inattention
Male C57BL/6 and Ccr2RFP/+Cx3cr1GFP/+ mice	45	10–14 weeks	Age	Tibial facture under isoflurane anesthesia	Glial activation, macrophage infiltration, cytokine release, and blood-brain barrier permeability
C57BL/6J mice	46	4 months	Age	Simple laparotomy under isoflurane anesthesia	Buried food test, open field test, and Y-maze test
C57BL/6J mice	47	2–8 months	Age	Simple laparotomy under isoflurane anesthesia	Open field test at 24 h following surgery plus anesthesia and increased a-synuclein and S100β
Female C57BL/6J mice	42	8–12 weeks	One microl of 10% w/v scrapie (ME7 strain)-infected C57BL6 brain homogenate	LPS	Food reward and escape from water motivated T-maze task
Male C57BL/6J mice	35	18–20 months	Age	Sevoflurane anesthesia, laparotomy and 12 h of ICU	Acute impairment of attention, memory, and thought organization; all mice were tested in Y-maze novel arm preference, buried food, attentional, and open field tests
Female mice	36	9–18 months	Age	Anesthesia and abdominal surgery	Delirium-like behavior assessed via buried food, open field, and Y-maze tests
C57BL/6-ME7 mice	39	Not stated	One microl of 10% w/v scrapie (ME7 strain)-infected C57BL6 brain homogenate	LPS, 100 μg/kg	T maze performed 12 weeks and 15 weeks post-disease initiation. Progressively diminished performance on T-maze test (fluctuating signs) due to progressive underlying degenerative features
MaleC57BL/6 mice	48	6–10 weeks	Age	*Streptococcus pneumoniae* pneumonia-induced sepsis	Behavioral tests include open field, Y-maze, elevated plus maze, Morris water maze, immediate shock deficit, cued and contextual fear conditioning
APPSwe/PS1dE9 mice	133	5–22 months	Double transgenic mice expressing a chimeric mouse/human amyloid precursor protein and a mutant human presenilin 1 (PS1-dE9), both directed to CNS neurons; both mutations are associated with early-onset Alzheimer's disease	100 μg/kg of LPS IP	LPS challenge acutely disrupted cognitive flexibility/executive function in Y-maze reversal learning tests and suppressed locomotor activity (performed at 15–16 months)
C57BL/6J Mice	134	24 months	Age	Poly I:C administration (12 mg/kg i.p.)	Acute disruption of working memory as measured by the "escape from shallow water" version of T-maze alternation in animals challenged with poly I:C
Per2-luck knock-in mice (C57BL/6J background)	32	4–8 months	Chronic disruption of circadian timing	LPS i.p. injection	Altered clock gene expression rhythms in suprachiasmatic nucleus, thymus, and peritoneal macrophages; no behavioral measures of anxiety/depression after circadian disruption
APPDwDI/mNos2$^{-/-}$ (CVNβAD) mice	43	3 and 12 month	AD-vulnerable brains (CVNβAD mice)	Tibial fracture surgery under isoflurane anesthesia	Failed resolution of inflammation, increased neurovascular vulnerability, hippocampal Aβ deposition, and impaired attention in 5-CSRTT

Model description (animal)	Reference	Age	Vulnerability factor	Precipitant	Model details/effects
Sprague-Dawley rats	[49]	14–16 weeks	None	Cardiopulmonary bypass with deep hypothermic circulatory arrest	Reduced microglia activation, reduced cell death, and improved neurological motor scores after treatment with Annexin A_1
Swiss Webster mice	[50]	3 month	Night time dim light exposure	LPS injection	Disrupted microglia inflammatory response, exaggerated sickness response with decreased home cage locomotor activity (on day following LPS)
C57BL/6J mice	[53]	99 weeks	Age	Polypharmacy diet	Declines in locomotor activity, rotarod latency, and front paw wire holding impulse

Abbreviations: CNS, central nervous system; LPS, lipopolysaccharide.

TABLE 4

Examples of rodent behavioral tests relevant to delirium

Human delirium feature	Rodent behavioral test examples[a]	References
Acute onset and fluctuating course	Assessment of behavior pre- and postinduction of delirium-inducing condition, home cage monitoring	32,34,50
Inattention	Spontaneous alternation (Y-maze), novel object interest/recognition, acoustic startle response, pre-pulse inhibition of startle response, 5-choice serial reaction time task, attentional set shifting	33,35,43,44,125
Disorganized thinking	Nest building, attentional set shifting task, T-maze[a]	23,33,35,39,40
Altered level of consciousness	Open field, novel object interest/recognition, acoustic startle response and prepulse inhibition of startle response, home cage monitoring	41,50,55,125
Memory impairment	Spontaneous alternation (Y-maze), T-maze, novel object interest/recognition, nest building, buried food test	35,36,38,40,55,125
Psychomotor activity (agitation or retardation)	Open field to assess time spent in center or periphery, elevated plus maze, home cage monitoring	50,125
Altered sleep-wake cycle	Home cage monitoring	50,55

[a]For several of the human delirium features, no one test currently exists that reliably informs the human delirium feature for which it is currently listed. We include these tests as potential examples for which a battery of tests is required to reveal the possibility of a common defect within the human delirium feature from expected behavior since the presence of a single test does not equate to the presence of a given delirium feature in rodents.

Alzheimers Dement. Author manuscript; available in PMC 2023 October 14.

TABLE 5

Candidate delirium biomarkers for animal model validation

	Humans		Animals		Human evidence for:		
Marker	**Human evidence**	**References**	**Evidence of causal effects in animal models**	**Refs**	**Risk markers**	**Disease markers**	**Outcome markers**
Biofluid Based							
Inflammatory							
• AGP	↑ CSF	145					
• CHI3L1/YKL-40	↑ Blood	146			X	X	
• Complement factor C3	↑ Blood, CSF	147					
• CCL2	↑ Blood	148				X	
• CRP	↑ Blood, CSF	149–151			X	X	
• FGF-21, FGF-23	↑ Blood	152				X	
• Gal-3BP	↑ Blood	153					
• IGF-1	↓ Blood	148,154				X	
• IL-1β	↑ Blood, CSF	155	Causal in multiple models			X	
• IL-1RA	↑ Blood, CSF	156,160	Protective in some models				
• IL-2	↑ Blood	157				X	
• IL-6	↑ Blood, CSF	156–162			X	X	
• sIL-6R	↑ Blood, CSF	163					
• IL-8	↑ Blood, CSF	162				X	
• IL-12	↑ CSF	164					
• MCP-1	↑ Blood	165				X	
• Natural killer (NK) cells	↑ Blood	166					
• Syndecan-1	↑ Blood	167				X	
• TNF-α	↑ Blood, CSF	168	Causal in some models		X	X	
Neurodegeneration or neuronal injury							
• Aβ	↑ CSF	169,170					
• GFAP	↑ Blood, CSF	170–173				X	

	Humans		Animals		Human evidence for:		
Marker	**Human evidence**	**References**	**Evidence of causal effects in animal models**	**Refs**	**Risk markers**	**Disease markers**	**Outcome markers**
• Neurogranin	↑ Blood	174				X	
• Neutrophil:Lymphocyte ratio	↑ Blood	175,176			X	X	
• NSE	↑ Blood	173,177,178				X	
• NfL	↑ Blood	170,171,179				X	
• P-tau	↑ CSF	180				X	
• PAI-1	↑ Blood	161					
• S100β	↑ Blood, CSF	162,178,181				X	
• T-tau	↑ Blood, CSF	170,180,182				X	
• UCHL1	↓ Blood	183				X	
Biochemical:							
• AChE	↓ Blood, CSF	168,183,184			X	X	
• Adrenaline	↓ CSF	176				X	
• Anticholinergic activity	↑ Blood, CSF	185	Disruption of relevantfunctions by cholinergic antagonists				
• BuChE	↓ Blood, CSF	186,187				X	
• Dopamine	↓ CSF	187				X	
• FA5	↑ CSF	188					
• Troponin	↑ Blood	175				X	
<u>Neurophysiological</u>							
• EEG—oscillatory dynamics	Relative power increase in slow frequencies and decrease in fast frequencies	87,89–102,189–192	↑ slow wave activity	107		X	
	—↑ θ						
	—↑ δ						
	—↓ β						
	—↓ α						
	And loss of high-frequency activity, especially in posterior brain regions					X	
	Altered α/θ, $\alpha/(\theta+\delta)$ or $(\alpha+\beta)/(\theta+\delta)$ ratio	94,101,192				X	
	Reduced peak frequency	101			X		

	Humans		Animals		Human evidence for:		
Marker	**Human evidence**	**References**	**Evidence of causal effects in animal models**	**Refs**	**Risk markers**	**Disease markers**	**Outcome markers**
	High preoperative alpha power	95			X	X	
• EEG—connectivity	↓ α band connectivity (possible marker for disturbed consciousness) and loss of directionality	94,102,103,191,192	↓ α band connectivity	107		X	
	↑ θ band connectivity (possible marker for attention/ memory deficits)	94,192				X	
	↓ β band connectivity (possible marker for instability of cognitive processes)	192				x	
	↑ δ band connectivity directed to frontal regions	191,192				X	
	Disturbance in default mode (DMN), salience (SN), and executive control (ECN) networks	192				X	
	Decrease in frontal-occipitoparietal connectivity	95				X	
• SSEP/BAEP	↑ Short- and long-latency sensory evoked potentials (SEP)	113				X	
Local field potentials (LFPs), Single neuron activity	↓ Hippocampal θ frequency; ↓ θ frequency and amplitude in medial prefrontal cortex; ↑ short-term δ frequency	118					
Imaging							
• Structural (MRI, DTI-MRI)							
	↑ Ventricular size (as a vulnerability marker)	193				X	
	New ischemic lesions, ↑ white matter hyperdensity/ disruption	194–198			X	X	X
	↓ Gray matter volume	196			X		
	↓ Brain volume	199					X
• Functional (fMRI)							
	↓ Connectivity strength (in aging and dementia)	103			X		
	↓ Efficiency and local clustering	103				X	
	Imbalanced connectivity in default mode network (DMN) and disrupted connectivity between DMN and dorsolateral prefrontal cortex (executive network)	74,200				X	
	Excessive striatum-salience network (anterior cingulate cortex and insula) connectivity	200				X	
	Disconnection of mesencephalic tegmentum, ventral tegmental area, and nucleus basalis with striatum/ thalamus	74,200	LPS-induced T-maze deficits reversed by peripheral glucose			X	X
• Functional (PET, SPECT)							

	Humans		Animals		Human evidence for:		
Marker	**Human evidence**	**References**	**Evidence of causal effects in animal models**	**Refs**	**Risk markers**	**Disease markers**	**Outcome markers**
	↓ Whole-brain glucose consumption, especially in posterior cingulate cortex	200		59			
	↓ Blood flow ratios in frontal and parietal cortex	201	↓ glucose consumption in neocortex			X	

Abbreviations: AChE, acetylcholinesterase; AGP, alpha-1-Acid glycoprotein; BuChE, butyryl cholinesterase; CRP, C-reactive protein; CSF, cerebrospinal fluid; FA5, coagulation factor V; GFAP, glial fibrillary acidic protein; IGF, insulin-like growth factor; IL, interleukin; CHI3L1/YKL-40, chitinase 3-like protein 1; fMRI, functional magenetic resonance imaging; Gal-3BP, galectin-3 binding protein; LPS, lipopolysaccharide; NfL, neurofilament light chain; MCP-1, monocyte chemoattractant protein-1; NSE, neuron-specific enolase; PAI-1, plasminogen activating inhibitor-1; R, receptor; PET, positron emission tomography; RA, receptor agonist; s, soluble; S100β, S100 calcium binding B; UCHL1, ubiquitin carboxyl-terminal-esterase-L1.

TABLE 6

Guidelines and recommendations for preclinical and translational models for delirium

Development of delirium model	• Choose species, include both sexes. • Choose delirium feature(s) to study, recognizing that high-fidelity models that mimic human delirium are not yet available. • Consider face and construct validity in development of model (ie, appropriate age, vulnerability, and precipitating factors [Table 2]) • Consider behavioral test(s) and frequency to mimic components of delirium in humans (Table 3). • Multiple animal models with different precipitants across different settings maybe required for cross-validation. • Carefully control for confounding factors (appetitive, motivational, locomotor, stress-related, illness-induced) that could be inappropriately conflated with delirium-like effects • Adhere to current standards for ethical treatment of animals
Experimental framework	• Appropriate blinding and randomization of outcome assessments • Reproducibility, which requires standardization of protocols and adequate sample sizes • Monitoringand physiological control of perturbations, especially anesthesia and infection • Standardization of behavioral protocols • Consideration of temporal alignment of pathophysiology and behavior • Cross-validation of behavioral changes with both human and animal biomarkers (eg, biofluid, EEG, imaging)
Validation of animal model	• Testing of animal responses to efficacious pharmacological and nonpharmacological human interventions
Reporting of animal studies	• Adherence to PREPARE guidelines[119] • Standardization and protocol validation • Data deposition in publicly accessible databases
Future research priorities	• Iterative testing and cross-translation of animal models and human delirium to optimize models, clarify pathophysiological mechanisms, and develop and test therapeutics • Large-scale collaborative studies to develop, standardize, and validate animal models for delirium; subsequent alignment with human studies as data become available

Abbreviation: EEG, electroencephalogram; PREPARE, Planning Research and Experimental Procedures on Animals: Recommendations for Excellence