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Enhancing Experimental Rigor and Reproducibility in Laboratory Animal Research on the Gut-Brain-Immune Axis

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ABSTRACT

Laboratory animal models are essential for investigating neuroimmune and systemic biological processes; however, experimental outcomes are often influenced by unrecognized biological and methodological variability. Increasing evidence suggests that coordinated dysfunction across multiple epithelial interfaces including gastrointestinal, dermal, and respiratory barriers represents an underappreciated source of variability in laboratory animal research. In standardized rodent studies, such barrier interdependence may contribute to systemic inflammation, neuroimmune priming, and inconsistent experimental findings. This narrative review synthesizes evidence from laboratory animal science and comparative pathology to propose a unified multi-barrier framework for interpreting experimental validity in gut-brain-immune (GBI) axis research. We examine how epithelial barrier disruption, metabolic and mitochondrial stress, and innate immune activation particularly sterile inflammatory pathways involving NLRP3 signaling interact to influence central nervous system homeostasis and commonly measured neuroimmune and behavioral outcomes. Emerging concepts, including epigenetic programming and glymphatic clearance, are discussed as integrative mechanisms linking peripheral physiological stress to central nervous system responses. The review also highlights environmental stressors, housing conditions, species- and strain-specific susceptibility, and perimortem tissue handling as important yet frequently overlooked variables in laboratory animal studies. Comparative observations from wild and captive species are considered as sentinel indicators of barrier vulnerability within a One Health framework. In addition, methodological refinements such as nano-enabled delivery platforms are discussed as tools that may improve dosing consistency and mechanistic interpretability. As a narrative review, the proposed framework is hypothesis-generating and remains conceptually oriented rather than causally established.

Keywords: Comparative pathology, Gut-brain-immune axis, Multi-barrier framework, Sterile inflammation (NLRP3 inflammasome).

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Introduction

The gut-brain-immune (GBI) axis has emerged as a central framework for understanding how peripheral physiology influences neural function, behavior, and disease susceptibility across animal species (Pereira et al., 2024). In laboratory animal research, GBI-focused models are increasingly employed to investigate neurodegenerative disorders, affective and cognitive dysfunction, metabolic disease, and immune-mediated pathologies (Müller & Di Benedetto, 2025). Yet the inherent sensitivity of these systems to environmental and physiological variability presents a significant challenge for experimental consistency and interpretation. Despite rapid expansion of this field, experimental findings often show

substantial variability, raising concerns regarding reproducibility and translational interpretation (Pereira et al., 2024). These challenges suggest that key biological variables influencing GBI regulation in laboratory animals remain incompletely defined (Müller & Di Benedetto 2025). Although this review focuses on GBI axis research, the principles discussed barrier interdependence, metabolic and immune stress, methodological variability, and staged validation are broadly applicable across laboratory animal science.

Current GBI research has predominantly focused on the intestinal barrier and gut microbiota as primary drivers of systemic and neuroimmune outcomes (Park et al., 2025). While this approach has yielded important insights, it may underestimate the contribution of other epithelial interfaces that are continuously exposed to environmental and experimental stressors (Missal et al., 2024).

This review introduces a unified multi-barrier framework, recognizing that intestinal, dermal, and respiratory barriers act as interconnected systems whose dysfunction can amplify systemic inflammation and neuroimmune variability (Lucas et al., 2024; Missal et al., 2024; O'Riordan et al., 2025; Park et al., 2025). Subsequent sections elaborate on this framework in relation to comparative pathology, metabolic stress, and sterile inflammation, highlighting its relevance for reproducibility and translational integrity in laboratory animal research.

Another emerging and timely area of scientific interest is the role of epigenetic programming in shaping long-term immune and neurobehavioral responses. Environmental stressors common to laboratory and captive animal environments can induce persistent epigenetic modifications affecting inflammatory signaling, mitochondrial function, and stress responsivity (Sajjanar et al., 2025; Sun et al., 2025). Such epigenetic priming may predispose animals to exaggerated or blunted responses in experimental models, contributing to inter-study variability and complicating data interpretation (Marei, 2025; Skinner, 2025). Incorporating epigenetic mechanisms into a multi-barrier GBI framework therefore provides a biologically grounded explanation for latent variability in laboratory animal phenotypes.

Importantly, these peripheral epigenetic imprints do not remain isolated but may converge on central nervous system homeostasis through neuroimmune clearance pathways. In particular, the glymphatic system has emerged as a critical interface linking peripheral inflammatory and metabolic disturbances with neuroinflammation and cognitive outcomes (Chen, Wang et al., 2025).

Recent work has expanded glymphatic research beyond sleep physiology and neurodegeneration to include sensitivity to systemic immune activation and metabolic stress hallmarks of epithelial barrier dysfunction (Bohr et al., 2022; Mogensen et al., 2021). Complementary evidence from rodent models further supports this peripheral–central coupling: Saihati et al., (2026) demonstrated that chronic exposure to proton pump inhibitors and H2 receptor antagonists induced neurocognitive decline in Wistar rats through systemic metabolic and signaling pathways, reinforcing the plausibility of barrier-linked mechanisms (Saihati et al., 2026). The glymphatic system has emerged as a promising translational endpoint in laboratory animal research, with rodent studies demonstrating its sensitivity to sleep, anesthesia, posture, and systemic inflammation. While further targeted animal model studies are needed to validate its role, glymphatic clearance provides a biologically plausible mechanism linking peripheral stressors to central outcomes.

From a One Health perspective, comparative pathology in wild and captive species provides valuable sentinel insights into how environmental stress, barrier disruption, and immune activation interact across biological systems (McGill, 2025). Integrating these observations with laboratory animal research offers a unique opportunity to identify conserved mechanisms that influence disease modeling and translational relevance (Wang et al., 2025). This comparative approach represents a further novel aspect of the review, positioning laboratory animals not as isolated systems but as part of a broader biological continuum (McGill, 2025; Wang et al., 2025).

While framed primarily through the lens of GBI axis research, the conceptual framework outlined here has broader implications for laboratory animal science. Barrier

dysfunction, metabolic stress, sterile inflammation, and methodological variability represent cross-cutting challenges in toxicology, neuroscience, immunology, and pharmacology. Recognizing and addressing these shared vulnerabilities may enhance experimental rigor, reproducibility, and animal welfare across disciplines.

As a narrative synthesis, this review draws on targeted PubMed and Web of Science searches guided by relevance to the multi-barrier framework, while acknowledging the inherent risks of selection bias and incomplete coverage. Building on this foundation, the review synthesizes current evidence from laboratory animal studies, comparative pathology, and emerging mechanistic research to propose a unified multi-barrier model of GBI axis dysfunction. By emphasizing epithelial barrier integrity, epigenetic priming, sterile inflammation, and neuro-immune clearance pathways, the review highlights several high-interest topics in contemporary biomedical science while maintaining a clear focus on laboratory animal research. Ultimately, this framework seeks to guide future experimental design, improve reproducibility, and enhance the translational validity of neuroimmune studies through refined interpretation of biological variability and animal-centered methodological rigor.

The Multi-Barrier Framework and Comparative Pathology in Laboratory Animal Research

Epithelial barriers represent the primary interfaces through which laboratory animals interact with their environment, experimental conditions, and microbial exposures. Increasing evidence indicates that dysfunction across these barriers is not isolated, but coordinated, with systemic consequences for immune activation, metabolic regulation, and neurophysiological outcomes.

The Multi-Barrier Framework: Coordinated Epithelial Dysfunction

Building on the multi barrier framework introduced in the Introduction, this section examines how comparative pathology highlights coordinated epithelial dysfunction and its implications for reproducibility.

Comparative Pathology and Experimental Reproducibility in Laboratory Animal Science

Comparative pathology across sentinel, captive, and laboratory species provides a powerful framework for identifying conserved mechanisms of barrier dysfunction and immune-metabolic dysregulation relevant to GBI research. Observations from nontraditional and sentinel species can reveal biological vulnerabilities that may remain latent or underappreciated in standardized rodent models, highlighting the value of broadening species selection beyond conventional laboratory rodents (Le Maho et al., 2025; Sikes & Paul, 2013; Suárez-Bonnet & Ramírez Rivero, 2023). By integrating findings from wild, captive, and laboratory animals, comparative pathology enhances the interpretation of experimental phenotypes and supports refinement of animal models, particularly by leveraging species specific insights into host physiology, immune responses, and disease progression (Groll et al., 2024; Mukherjee et al., 2022).

Quantitative estimates of the prevalence of subclinical barrier dysfunction in laboratory rodent facilities remain scarce, and available data are largely anecdotal or facility specific; this gap highlights the need for systematic surveillance.

Sentinel Species and Barrier Vulnerability

Sentinel species exposed to environmental stressors often manifest early or exaggerated forms of epithelial barrier dysfunction, providing insight into systemic immune activation mechanisms relevant to laboratory mammals. Pathological findings in *Spalax leucodon* (Egyptian mole rat), for example, demonstrate how chronic dermal insult such as parasitic perifollicular dermatitis can function as a primary immunological stressor. Field studies have identified mixed ectoparasitic infestations, including *Lynxacarus egyptiacus* and *Polyplax serrata*, resulting in keratinolysis and perifollicular inflammation with prominent lymphocytic infiltration (Tamam, 2014). Importantly, dermal barrier disruption in these animals is frequently accompanied by secondary pathology at distal epithelial interfaces.

Evidence directly linking dermal barrier dysfunction to GBI outcomes in laboratory rodents remains limited; this section is therefore framed as hypothesis generating, supported by comparative pathology and correlative observations.

Similar patterns are observed in sentinel carnivores such as the cheetah (*Acinonyx jubatus*), where environmental and management-associated stressors are linked to chronic gastritis and lymphoplasmacytic enteritis, systemic immune dysregulation, and increased susceptibility to opportunistic respiratory infections (Terio et al., 2005). Across sentinel taxa, epithelial barrier failure is rarely localized; instead, it reflects circulating inflammatory mediators and sterile inflammatory signaling that compromise multiple mucosal surfaces simultaneously (O'Riordan et al., 2025).

Although Spalax is not a conventional laboratory species, comparable patterns of barrier interdependence have been reported in rodents subjected to chronic stress, suboptimal housing, or immune challenge. While these parallels are primarily supported by review level observations, they provide hypothesis generating insights into how sentinel species pathology may inform laboratory rodent research. In laboratory mice and rats, dermal irritation, respiratory mucosal inflammation, and intestinal dysbiosis frequently co-occur under conditions such as overcrowding, altered bedding, or environmental instability. These parallels underscore the translational relevance of sentinel species pathology and support its use as a hypothesis-generating framework for understanding barrier-driven variability in laboratory animal research.

Implications for Experimental Reproducibility

The convergence of epithelial barrier disruption, metabolic stress, and immune activation across species highlights the importance of considering systemic pathology when interpreting laboratory animal data. Failure to recognize subclinical barrier dysfunction or metabolic compromise may contribute to unexplained variability in neurobehavioral, inflammatory, or therapeutic outcomes. In this context, comparative pathology functions not only as a descriptive discipline but also as a hypothesis-generating tool for identifying hidden covariates in experimental design (Ardicli et al., 2024). By situating laboratory findings within a broader comparative framework, researchers can better distinguish conserved biological responses from model-specific artifacts, thereby strengthening reproducibility, translational relevance, and animal welfare (Lange & Inal, 2024).

Key examples of epithelial barrier dysfunction across species and their relevance to laboratory animal models are summarized in Table 1.

Metabolic and Mitochondrial Pathology as Upstream Drivers of Sterile Inflammation

Comparative pathological studies in wild and captive mammals, including *Acinonyx jubatus* (cheetah), further highlight the role of metabolic dysregulation in systemic inflammation. In cheetahs, captivity associated stressors such as altered diet, restricted exercise, and psychosocial stress have been linked to a high prevalence of chronic lymphoplasmacytic gastritis associated with *Helicobacter spp.*, despite similar colonization patterns in wild populations, underscoring the influence of environmental and metabolic stress on barrier pathology and immune activation (Mangiaterra et al., 2022; Terio et al., 2005). Captive cheetah pathology also frequently involves hepatic alterations, including amyloid deposition and other signs of systemic metabolic strain, suggesting a broader metabolic syndrome like phenotype in affected individuals.

Published evidence from metabolic stress models across species demonstrates that high fat diets, chronic inflammation, and related metabolic challenges induce hepatic steatosis and focal necrosis, accompanied by pronounced mitochondrial dysfunction characterized by altered oxidative phosphorylation, increased reactive oxygen species production, and the release of mitochondrial damage associated molecular patterns (mtDAMPs) that amplify inflammatory signaling pathways (Ding et al., 2023; Guan et al., 2025). Importantly, similar metabolic and mitochondrial features are observed in laboratory rodents subjected to high fat diets or experimental inflammatory stress, where systemic low grade inflammation, mitochondrial impairment, and dysregulated metabolic signaling contribute to multi organ pathology including hepatic and adipose (Elshareif et al., 2023). These parallels reinforce the translational relevance

of comparative metabolic pathology to laboratory animal research and highlight mitochondria as central hubs linking metabolic dysregulation to immune activation across species.

Mitochondrial dysfunction is of particular relevance to laboratory animal models, as impaired mitophagy can result in the cytosolic release of mitochondrial DNA (mtDNA), a potent activator of innate immune pathways such as cGAS–STING signaling (Decout et al., 2021). In parallel, metabolic by-products such as urate crystals and lipid-rich deposits classically observed in conditions including avian visceral gout and hepatic steatosis serve as macro-pathological indicators of NLRP3 inflammasome activation (Warda et al., 2025). Activation of these pathways has been implicated in sterile inflammation, neuroimmune priming, and altered behavioral outcomes in rodent models of metabolic stress and chronic inflammation, reinforcing the translational value of comparative metabolic pathology for interpreting laboratory animal phenotypes (O'Riordan et al., 2025). Such conserved metabolic-mitochondrial responses across species highlight the utility of comparative pathology for anticipating immune and behavioral phenotypes in laboratory rodents subjected to metabolic or inflammatory challenge.

Collectively, conserved metabolic and mitochondrial stress responses across species suggest that peripheral immune–metabolic dysregulation may exert effects beyond local tissues, with implications for central nervous system homeostasis. Sterile inflammation driven by mitochondrial dysfunction, inflammasome activation, and circulating metabolic mediators provides a plausible mechanistic link between peripheral pathology and neuroimmune outcomes frequently assessed in laboratory animal models. These systemic signals may influence cerebrovascular dynamics, immune priming, and waste clearance mechanisms, positioning central clearance pathways as downstream integrators of peripheral stress.

Table 1.

Comparative pathology of epithelial barrier dysfunction across species and relevance to laboratory animal models.

Species / Model	Primary Barrier Affected	Key Pathology Observed	Relevance to Laboratory Animal Models	References
Spalax leucodon	Dermal → Respiratory	Parasitic perifollicular dermatitis; secondary mycotic granulomas	Highlights barrier interdependence; parallels stress-induced dermal and respiratory inflammation in rodents	(Tamam, 2014)
Acinonyx jubatus	Metabolic / Hepatic	Mitochondrial swelling; nucleolar fragmentation; impaired mitophagy	Models captive stress and metabolic dysregulation relevant to diet- and inflammation-induced rodent studies	(Tordiffe, 2017)
Laboratory mouse (*Mus musculus*)	Intestinal / Systemic	Dysbiosis; increased gut permeability; sterile inflammation	Core GBI model; influenced by housing, diet, and handling	(Okumura & Takeda 2024; Stolfi et al., 2022)
Laboratory rat (*Rattus norvegicus*)	Respiratory / Metabolic	Mucosal inflammation; oxidative stress	Relevant to inhalation studies, anesthesia effects, and chronic stress paradigms	(Rothenburger et al., 2015; Wiriansya et al. 2023)

Notes: GBI, gut-brain-immune axis; Mus musculus, laboratory mouse; Rattus norvegicus, laboratory rat; Spalax leucodon, blind mole rat; Acinonyx jubatus, cheetah.

Glymphatic Function and Neuroimmune Homeostasis in Laboratory Models

The glymphatic system has emerged as a critical pathway for cerebrospinal fluid-interstitial fluid exchange and metabolic waste clearance in the central nervous system, primarily characterized in rodent models (Iliff et al., 2012). Experimental studies in mice and rats have demonstrated that glymphatic activity is strongly influenced by physiological states commonly manipulated in laboratory settings, including sleep-wake cycles (Xie et al., 2013), body posture (Lee et al., 2015), circadian rhythms, and anesthetic regimens (Gakuba et al., 2018). These findings underscore the relevance of glymphatic function to laboratory animal research, particularly in studies assessing neuroinflammation, cognition, and neurodegeneration.

Sleep has been identified as a major modulator of glymphatic clearance, with slow-wave sleep facilitating increased interstitial space and enhanced convective fluid flow (Hauglund & Nedergaard, 2025). Conversely, sleep deprivation and fragmentation impair glymphatic efficiency and alter aquaporin-4 expression, leading to reduced clearance of neurotoxic metabolites (Vasciaveo et al., 2023). Recent mechanistic work further demonstrates that norepinephrine oscillations during natural sleep actively regulate glymphatic clearance, clarifying why anesthesia produces distinct clearance dynamics compared to physiological sleep (Martini, 2025). Indeed, commonly used anesthetics in rodent research differentially affect glymphatic flow, highlighting anesthesia as a potential confounding variable in neurobiological experiments (Gakuba et al., 2018). In addition to sleep and anesthesia, body posture also modulates glymphatic transport, with lateral positions enhancing clearance compared to supine (Lee et al., 2015).

Beyond central nervous system-specific factors, emerging evidence suggests that peripheral physiological states may indirectly influence glymphatic function. Systemic inflammation, metabolic stress, and altered vascular dynamics conditions frequently observed in experimental disease models and under chronic stress have the potential to modify cerebrovascular pulsatility and fluid exchange (Krings et al., 2025). In this context, it is plausible that systemic metabolic dysregulation and sterile inflammatory signaling may influence the pressure gradients necessary for effective glymphatic clearance, although direct causal relationships remain to be established (Chen, Wang et al., 2025).

Comparative pathology further supports the relevance of this conceptual linkage. Metabolic disorders and inflammatory conditions in animal models, including hepatic dysfunction and peripheral metabolic stress, have been shown to contribute to neuroinflammatory cascades and alterations in central metabolism and behavior in controlled studies of obesity and liver-brain axis dysfunction (Asimakidou et al., 2025). Mitochondrial dysfunction has been mechanistically tied to chronic inflammation and systemic metabolic imbalance, with pathways such as JAK2/STAT3 activated in hyperuricemia-induced stress models (Kim et al., 2025). While these observations do not confirm impaired glymphatic function per se, emerging reviews emphasize the importance of exploring how metabolic dysregulation may disrupt glymphatic clearance and peripheral–central coupling mechanisms (Chen, Meseguer et al., 2025).

These findings position the glymphatic system as a potential integrative node linking peripheral immune and metabolic status to central nervous system homeostasis in laboratory animals (Chen, Meseguer et al., 2025; Xu et al., 2025). Experimental and clinical studies increasingly suggest that glymphatic transport is sensitive to systemic metabolic disturbances and peripheral inflammatory signals, thereby influencing neuroimmune balance and waste clearance within the brain (Wang et al., 2023). Framing glymphatic dysfunction as a modifiable and experimentally testable variable may therefore enhance the interpretation of neuroimmune outcomes and support refinement of experimental design in glymphatic brain interaction related research, particularly in disease and injury models where systemic and central processes converge (Lopes et al., 2024). While mechanistically compelling, direct experimental evidence connecting peripheral epithelial barrier dysfunction to impaired glymphatic clearance is sparse, and this discussion is intended as a conceptual model requiring further validation.

Limitations of Molecular Readouts in Assessing Biological Function

Interpretation of experimental outcomes in GBI research frequently relies on molecular readouts such as gene and protein expression; however, these measures do not necessarily reflect functional biological activity. Transcript abundance often correlates poorly with protein levels due to post transcriptional regulation, variable translational efficiency, and protein degradation dynamics (Liu et al., 2016). Moreover, protein detection alone does not guarantee biological functionality, as many proteins require specific post translational modifications, appropriate subcellular localization, or interaction with cofactors to become active (Kim et al., 2006). Even signaling pathway activation can be context dependent, as demonstrated by site specific differential activation of Ras/Raf/ERK signaling in rabbit isoproterenol induced left ventricular hypertrophy (Kim et al., 2025). Commonly used techniques such as Western blotting typically quantify total protein abundance but do not capture enzymatic activity, conformational state, or regulatory modification status. Consequently, proteins may be highly expressed yet functionally inactive or constrained by contextual variables such as metabolic state, inflammatory tone, or cellular stress. Failure to account for these layers of regulation may lead to incorrect mechanistic conclusions, particularly in laboratory animal models where systemic stress, mitochondrial dysfunction, and sterile inflammation are prevalent sources of biological variability.

Bridging the Gap: Sterile Inflammation as a Systems-Level Confounder

Within the multi barrier framework outlined earlier, this section explores sterile inflammation as a systems level confounder, focusing on how barrier dysfunction interacts with metabolic and mitochondrial stress to bias neuroimmune readouts.

Innate Immune Sensing and Sterile Inflammation in Laboratory Animal Models

As a background confounder, baseline immune activation shapes disease phenotypes in models of metabolic stress, neuroinflammation, and immune-mediated pathology. Endogenous danger signals (DAMPs) activate pathways such as the NLRP3 inflammasome, underscoring how systemic stress can bias experimental readouts. Among the innate immune pathways implicated in this process, the NLRP3 inflammasome has been extensively studied in laboratory rodents and is increasingly recognized as a central regulatory node linking peripheral pathology to systemic immune activation (Konopko et al., 2025; Pinzón-Fernández et al., 2025).

In mouse models, activation of NLRP3 has been shown to occur in response to diverse stimuli relevant to laboratory animal research, including extracellular ATP, urate crystals, mitochondrial reactive oxygen species, and cytosolic mitochondrial DNA. Genetic studies using Nlrp3-deficient mice, as well as animals lacking downstream effectors such as caspase-1 or IL-1β, have demonstrated attenuated inflammatory responses in models of metabolic syndrome, neuroinflammation, and stress-induced behavioral alterations. These findings highlight the utility of inflammasome-deficient animals for dissecting immune contributions to experimental phenotypes and underscore the translational relevance of sterile inflammation pathways in laboratory research.

Importantly, NLRP3 signaling does not operate in isolation. Crosstalk between mitochondrial dysfunction, impaired autophagy, and innate immune sensing has been repeatedly documented in laboratory rodents (Hu et al., 2024). Failure of mitophagy a phenomenon observed under conditions of chronic stress, high-fat diet exposure, or toxic insult can lead to the accumulation of damaged mitochondria and release of mitochondrial DNA, thereby amplifying inflammasome activation (Wu et al., 2025). This mechanistic coupling is particularly relevant in laboratory animal settings, where metabolic load and environmental stressors may vary subtly yet significantly between facilities or experimental protocols (Weber et al., 2025).

Housing conditions, diet composition, microbiota status, and repeated handling have all been reported to influence inflammasome activity in rodents, potentially contributing to inter-study variability (Wu et al., 2025). While NLRP3 serves as an informative mechanistic target, it should not be regarded as a singular or universal driver of pathology; rather, its activation reflects the integrated physiological state of the animal (Weber et al., 2025).

Limitations and Integrative Perspectives on Sterile Inflammation in Laboratory Animal Models

Several limitations warrant consideration. First, not all hidden systemic stress signal responses in laboratory animals are NLRP3-dependent, as parallel pathways involving AIM2, NLRC4, or cGAS-STING signaling may predominate depending on tissue context and experimental design (Swanson et al., 2019). Second, the majority of mechanistic insights derive from inbred mouse strains, which may not fully capture the heterogeneity observed across species or housing conditions (Weber et al., 2025). These constraints underscore the importance of complementary approaches and cautious extrapolation when interpreting inflammasome biology in experimental systems.

Hence, viewing NLRP3 as a central regulatory node rather than a master switch provides a more nuanced framework for interpreting sterile inflammation in laboratory animal models. Integrating inflammasome biology with barrier integrity, metabolic state, and environmental variables may improve experimental design, enhance reproducibility, and refine the translational relevance of GBI-focused research (Hu et al., 2024; Wu et al., 2025).

Together, these observations underscore how basal sterile inflammation may act as an unrecognized background variable in laboratory animal studies, reinforcing the need for methodological strategies that improve exposure control and mechanistic interpretability.

It is important to note, however, that NLRP3 represents only one of several stress responsive pathways. Neuroendocrine mechanisms such as HPA axis activation and glucocorticoid signaling can independently modulate immune tone and contribute to inter individual variability in GBI outcomes, underscoring the need for a balanced view of regulatory nodes.

Nano-Enabled Delivery Platforms as Preclinical Research Tools in Laboratory Animal Models

Against this background of biological and methodological variability, nano-enabled delivery platforms have emerged as experimental tools aimed at refining exposure control and improving interpretability in laboratory animal studies. In the context of GBI research, these approaches are best viewed not as therapeutic solutions, but as preclinical formulation strategies designed to reduce dosing variability and formulation-related confounding.

Stability, Bioavailability, and Reproducible Dosing Variability in Rodent GBI Models

Nano-enabled delivery platforms are increasingly explored in laboratory animal research as methodological tools to address persistent challenges related to compound stability, bioavailability, and dosing variability. These limitations are particularly relevant in studies of the gut-brain-immune (GBI) axis, where experimental outcomes are highly sensitive to differences in gastrointestinal exposure, microbial metabolism, and host immune priming (Banerjee et al., 2026). Variability in oral delivery can therefore introduce substantial experimental noise, complicating interpretation of mechanistic relationships across barrier systems.

In rodent models, inconsistent delivery of bioactive compounds to their intended sites of action represents a major experimental constraint. Many molecules relevant to neuroimmune and microbiome research are susceptible to degradation by gastric acidity, bile salts, and proteolytic enzymes, or undergo extensive first-pass metabolism (Vijayaram et al., 2025). Peptide-like agents, including bacteriocins and certain postbiotics, are particularly vulnerable to proteolysis, while several dietary or microbial derived neuroactive metabolites exhibit limited intestinal uptake and rapid systemic clearance (Banerjee et al., 2026). Such pharmacokinetic instability can obscure dose-response relationships and contribute to poor reproducibility across studies.

These pharmacokinetic constraints can result in substantial inter-animal variability despite standardized dosing protocols, complicating the interpretation of behavioral, immunological, and neuroinflammatory endpoints and contributing to inconsistencies across laboratories (Hu et al., 2024). From a laboratory animal science perspective, formulation strategies that enhance compound stability and standardize release profiles may therefore improve experimental reproducibility and reduce unnecessary animal use by decreasing outcome variability (Hu et al., 2024; Wu et al., 2025).

Nano-Enabled Formulation Strategies as Experimental Refinement Tools in Rodent GBI Research

Within the broader experimental landscape of GBI research, psychobiotics and related bioactives including specific probiotic-derived metabolites, postbiotics, bacteriocins, amino acid derivatives, and small neuroactive compounds serve as a relevant and timely case study. Many of these agents are biologically active at low concentrations yet exhibit poor stability in the gastrointestinal tract, variable absorption, or rapid degradation, limiting consistent exposure in laboratory animal experiments (Vijayaram et al., 2025). Consequently, nano-enabled delivery systems (e.g., nanoemulsions, lipid-based carriers, polymeric or encapsulation-based platforms) serve as viewed primarily as preclinical formulation strategies aimed at improving exposure control and mechanistic interpretability in laboratory animal studies, rather than as clinical-ready therapeutic solutions (Banerjee et al., 2026).

Within this context, nano-enabled formulation strategies offer a controlled experimental approach to improve delivery precision and exposure consistency in laboratory rodents. Importantly, their value in GBI-axis research serve as viewed primarily as a methodological refinement rather than a therapeutic intervention, enabling more reliable targeting of intestinal, immune, or systemic compartments while reducing formulation-related variables (Sato et al., 2023). When applied under well-defined experimental conditions, these platforms can strengthen mechanistic inference by improving dose accuracy, temporal control of release, and tissue-specific bioavailability.

Consequently, nano-enabled delivery systems serve as considered part of an integrated experimental design framework aimed at enhancing reproducibility and translational validity in barrier-focused research. By reducing exposure heterogeneity and pharmacokinetic uncertainty, they may help clarify causal relationships within the GBI axis and improve cross-study comparability without implying intrinsic therapeutic superiority.

Lipid-based nanoemulsions, including medium-chain triglyceride based systems, are among the most widely used platforms in rodent studies due to their relative biocompatibility and ease of formulation. These systems may improve the solubility and stability of poorly water-soluble or acid-labile compounds, thereby reducing degradation during gastric transit and facilitating more predictable intestinal exposure. In laboratory rodent experiments, such improvements can help minimize inter-animal variability attributable to inconsistent dosing or compound instability, which is particularly relevant in longitudinal GBI studies. Insights from comparative lipid research for example, the influence of phospholipid and fatty acid composition on vesicle properties in camel erythrocyte membranes (Warda & Zeisig 2000) underscore the broader principle that lipid architecture critically shapes carrier stability and bioactive delivery.

Mucoadhesive and pH-responsive delivery systems provide additional opportunities to interrogate region-specific gastrointestinal processes in laboratory rodents. By enabling preferential release in defined segments of the gastrointestinal tract, these carriers can support mechanistic investigations of mucosal immunity, epithelial barrier integrity, microbial ecology, and localized inflammatory signaling. Functional nanocarriers with mucopenetrating and mucoadhesive properties have been identified as promising approaches for modulating drug absorption in the gastrointestinal tract by prolonging residence time near the epithelial surface and enhancing controlled exposure (Sato et al., 2023).

Encapsulation strategies for postbiotics, microbial metabolites, or small bioactive molecules may further enhance experimental reproducibility by improving formulation stability and reducing batch-to-batch variability. This consideration is particularly important in multi-week or longitudinal rodent studies, where compound degradation during storage or repeated preparation can introduce unintended variability that obscures dose-response relationships and complicates the interpretation of neuroimmune or behavioral outcomes. Improved formulation stability can therefore contribute to more reliable comparisons across experimental groups and time points (Kamble et al., 2025).

In the context of psychobiotic and GBI-focused research, nano-enabled formulations can facilitate hypothesis testing under more controlled exposure conditions. By limiting variability related to compound solubility, degradation, or inconsistent gastrointestinal delivery,

these platforms help strengthen causal inference without conflating formulation effects with intrinsic biological efficacy (Banerjee et al., 2026). When incorporated within rigorous experimental designs and appropriate controls, nano-enabled strategies may thus serve as methodological refinements that enhance reproducibility, interpretability, and translational relevance in laboratory rodent models of GBI interaction (Vijayaram et al., 2025).

Together, these sources of biological and procedural variability underscore the need for integrated refinement strategies that not only strengthen the rigor of experimental design but also uphold ethical considerations in laboratory animal research. As summarized in Table 2, such strategies should encompass standardized housing and handling protocols, careful dietary formulation, transparent reporting of species- and strain-specific susceptibilities, and refined tissue collection procedures that minimize perimortem stress and blood-related artifacts. By systematically addressing these methodological variables, researchers can minimize latent sources of experimental variability, improve reproducibility across studies, and enhance the translational validity of gut-brain-immune axis investigations, while simultaneously reinforcing principles of animal welfare and responsible scientific practice.

Table 2.

Key Methodological Considerations Influencing GBI Axis Research in Laboratory Animals.

Consideration	Impact on GBI Axis Research	Strategic Refinement	Supporting References
Handling Stress	Triggers neuroendocrine–immune priming; alters gut permeability and neuroimmune signaling	Habituation and standardized handling protocols	Bailey et al., 2011; Moradian et al., 2024; Morys et al., 2024
Housing Conditions	Modulates gut microbiota composition, epithelial barrier integrity, and stress responsiveness	Optimized housing density and environmental stability	Bailey et al., 2011; Park et al., 2025
Tissue Collection	Residual intravascular blood inflates inflammatory and metabolic readouts	Controlled euthanasia and vascular perfusion where appropriate	National Research Council (US) Institute for Laboratory Animal Research, 1996
Nano-Enabled Delivery	Reduces pharmacokinetic variability; may introduce formulation-driven immune effects	Validated MCT-based or lipid nano-encapsulation with vehicle controls	Dinan & Cryan, 2017; Sarkar et al., 2016; Singh & Lillard, 2009
Species Selection	Captures species- and strain-specific biological vulnerability relevant to GBI outcomes	Use of biologically justified models and sentinel species data (e.g., *Spalax*, cheetah)	Park et al., 2025; Tamam, 2014.

Notes: GBI, gut-brain-immune axis; MCT, medium-chain triglycerides.

Ethical and Experimental Refinement in Laboratory Animal Science

Ethical and experimental refinement are closely linked determinants of rigor and reproducibility in laboratory animal research. This section considers how these principles intersect in the design and interpretation of GBI-focused studies.

Experimental and Ethical Considerations in Laboratory Animal Studies

Nano-enabled formulations introduce additional layers of experimental complexity that must be explicitly addressed in laboratory animal research. Beyond the intended biological activity of the active compound, formulation components themselves including surfactants, lipid carriers, and particulate structures may influence immune signaling, epithelial barrier integrity, stress responsivity, or gut microbiota composition. If inadequately controlled, these formulation-driven effects can confound interpretation and lead to misattribution of biological outcomes (Dinan & Cryan, 2017; Sarkar et al., 2016; Singh & Lillard, 2009).

Accordingly, studies employing nano-enabled delivery systems should incorporate rigorous experimental controls and transparent reporting practices. These include the use of vehicle-only nano-carrier control groups, verification of dose stability and recovery in feed, water, or gavage preparations, and monitoring for non-specific inflammatory, metabolic, or stress-related responses (Park et al., 2025). Detailed characterization of formulation composition, particle size distribution, and storage conditions is also essential to ensure reproducibility and interpretability across laboratories.

Importantly, such formulation-level rigor serves as viewed not merely as a technical requirement but as a form of experimental refinement. By reducing ambiguity and minimizing unnecessary animal use arising from inconclusive or misleading data, careful control of nano-enabled variables directly supports ethical principles within laboratory animal science and aligns with international expectations for responsible experimental design (Azkona, 2023; Devan et al., 2024).

Ethical and Experimental Considerations within a One Health Framework

Laboratory animal science increasingly recognizes that ethical animal care and experimental validity are inseparable. In studies of the GBI axis, biological systems are particularly sensitive to environmental and procedural variables, including housing conditions, handling practices, species and strain selection, and tissue collection methods. These factors exert measurable effects on immune regulation, epithelial barrier integrity, microbiota composition, and neurobehavioral outcomes, making them central determinants of both animal welfare and scientific rigor (Bailey et al., 2011; Moradian et al., 2024; Morys et al., 2024).

From a One Health perspective, stress exposure, environmental instability, and inappropriate model selection not only compromise animal well-being but may also distort experimental outcomes in ways that undermine translational relevance. Chronic or acute stress can prime immune responses, alter gut permeability, disrupt circadian rhythms, and influence neuroimmune signaling, thereby mimicking or masking experimental interventions (Park et al., 2025). Similarly, species- and strain-specific susceptibilities shaped by differences in epithelial barrier architecture, immune tone, and neuroendocrine regulation must be carefully matched to research questions to avoid overgeneralization or misinterpretation.

Ethical refinement in this context extends beyond compliance with welfare guidelines to encompass methodological decisions that reduce biological noise and improve interpretability. Standardized handling, appropriate environmental enrichment, transparent reporting of housing and husbandry conditions, and careful consideration of euthanasia and tissue collection procedures all contribute to minimizing stress-induced bias and procedural artifacts (Morys et al., 2024; National Research Council (US) Institute for Laboratory Animal Research, 1996). When such factors are systematically addressed, laboratory animal models more accurately reflect biologically meaningful processes rather than experimental contingencies.

Integrating these ethical and experimental considerations within a One Health framework reinforces the translational integrity of GBI-axis research. By aligning animal welfare, methodological rigor, and cross-species biological insight, laboratory animal science can better ensure that observed outcomes represent conserved biological mechanisms rather than artifacts of experimental design (Moradian et al., 2024; Park et al., 2025).

Refinement Strategies to Reduce Bias and Improve Reproducibility

Reducing experimental bias and improving reproducibility in laboratory animal research requires the deliberate application of refinement strategies, particularly in GBI-focused studies where biological variability is pronounced.

Refinement of Animal Models and Experimental Procedures

Refinement strategies aimed at minimizing stress and physiological disturbance are central to GBI axis research, where neuroendocrine and immune pathways are highly sensitive to environmental and procedural variables (Bailey et al., 2011; Moradian et al., 2024). Handling procedures, euthanasia methods, and housing conditions can rapidly

activate stress-responsive signaling cascades, potentially obscuring mechanistic interpretation if not carefully controlled. Optimizing housing density, maintaining stable circadian light-dark cycles, and implementing habituation protocols have been shown to reduce stress-induced immune priming and improve consistency across experimental groups (Morys et al., 2024).

Equally important is the biologically informed selection of animal species and strains. Model choice based on known species- and strain-specific susceptibilities such as differences in epithelial barrier integrity, immune responsiveness, and stress reactivity enhances experimental validity and reduces unnecessary animal use by aligning biological predisposition with research objectives (Park et al., 2025). Existing reports provide case specific demonstrations of refinement benefits, but systematic quantitative comparisons across strategies remain lacking.

Reduction of Stress- and Procedure-Induced Experimental Bias

Beyond preventive refinement, both chronic and acute stressors represent significant yet often underappreciated sources of experimental bias in laboratory animal studies. Perimortem stress associated with handling or euthanasia can induce rapid surges in glucocorticoids and catecholamines, altering immune signaling, gene expression, vascular permeability, and metabolic dynamics within minutes (Bailey et al., 2011; Park et al., 2025).

When combined with tissue collection methods that retain residual intravascular blood particularly in highly vascularized organs such as the liver, spleen, lungs, and brain these effects may artificially inflate inflammatory or metabolic markers, confounding interpretation of tissue-resident processes (National Research Council (US) Institute for Laboratory Animal Research, 1996). Careful selection of euthanasia techniques, attention to perfusion status where compatible with study aims, and transparent reporting of procedural details are therefore critical components of experimental refinement and reproducibility.

Environmental Enrichment as a Biological Modulator

Environmental enrichment offers a dual benefit in laboratory animal research: it supports animal welfare while actively modulating biological systems directly relevant to the GBI axis. Enrichment strategies influence stress resilience, immune balance, microbiota diversity, and neuroplasticity processes that intersect with epithelial barrier function and neuroimmune regulation (Moradian et al., 2024; Morys et al., 2024).

When systematically applied and clearly reported, environmental enrichment is regarded as a biologically meaningful component of experimental design rather than an uncontrolled confounder. Treating enrichment as a controlled variable enables more nuanced interpretation of neuroimmune and behavioral outcomes while simultaneously supporting ethical refinement.

Nano-Enabled Delivery as a Methodological Refinement Strategy

Discussion of nano enabled delivery systems is included here as an illustrative example of methodological refinement, highlighting how formulation consistency and vehicle controls can influence reproducibility within the multi barrier framework. From a laboratory animal science perspective, nano-enabled delivery strategies including those applied to psychobiotic-related compounds are regarded as experimental formulation tools rather than clinical-ready interventions (Dinan & Cryan, 2017; Sarkar et al., 2016; Singh & Lillard, 2009). Their principal value lies in improving exposure precision, reducing dosing variability, and strengthening mechanistic inference in GBI-focused laboratory animal studies, rather than in presumed therapeutic superiority.

When implemented with appropriate experimental controls and transparent reporting, nano-enabled formulations can reduce formulation-driven variability associated with conventional oral delivery and enhance the interpretability of neuroimmune and behavioral endpoints. Viewed in this context, nano-enabled approaches function as methodological refinements that support reproducibility and ethical animal use, without conflating formulation effects with intrinsic biological efficacy.

Staged Validation Frameworks for Experimental Rigor

Strengthening experimental rigor in laboratory animal research requires validation strategies that extend beyond isolated molecular readouts and single-stage testing, particularly in complex systems such as the GBI axis.

Methodological Perspectives: Integrating Omics, In Silico Modeling, and Staged Validation in Laboratory Animal Research

The interpretability and reproducibility of laboratory animal studies particularly in GBI axis research depend not only on experimental design but also on the extent to which molecular readouts reflect true biological function. As emphasized throughout this review, barrier dysfunction, metabolic stress, and sterile inflammation introduce layers of physiological variability that can influence neuroimmune and behavioral outcomes if not adequately controlled. Reliance on isolated gene or protein expression data without functional validation therefore risks oversimplifying complex, system-level processes. Taken together, these limitations underscore the need for a structured validation strategy that places molecular observations within a broader functional and physiological context before they are interpreted in vivo. To address these challenges, a staged methodological framework integrating high-resolution omics, in silico modeling, and in vitro validation is increasingly recognized as essential. Such approaches function as critical upstream filters prior to in vivo experimentation. Within laboratory animal science, and particularly in studies employing nano-enabled delivery concepts, such structured validation pipelines align with international standards for Replacement, Reduction, and Refinement (3Rs) by ensuring that only biologically plausible, stable, and mechanistically informed interventions advance to animal testing (Devan et al., 2024). Early-stage validation not only screens candidate formulations but also refines mechanistic hypotheses and reduces the likelihood of inconclusive or misleading animal data, thereby addressing a central source of experimental variability highlighted in GBI research (Park et al., 2025). In this context, in silico approaches including molecular docking, molecular dynamics simulations, and systems-level computational modeling serve as vital refinement tools, enabling the generation of high-confidence, testable hypotheses regarding interactions between environmental toxicants, bioactive compounds, and key physiological signaling pathways before or alongside in vivo trials.

High-Resolution Omics as a Foundation for Phenotypic Contextualization

Within such staged validation pipelines, omics-based approaches represent a logical first step, as they provide an integrated, systems-level snapshot of physiological state prior to targeted mechanistic testing.

Omics-based profiling, particularly proteomics and glycomics, provides a systems-level perspective on physiological states that extends beyond transcriptomic inference. In the context of laboratory animal science, these tools are essential for identifying the "molecular interaction" occurring within an animal subjected to experimental stressors.

Proteomic analyses enable the identification of stress-response signatures and mitochondrial dysfunction that collectively shape barrier integrity. For instance, proteomic analysis serves as a critical indicator of cellular battle against oxidative stress and metabolic failure (Kim et al., 2006). Such integrated molecular patterns are vital for validating that a laboratory model truly reflects the physiological processes under investigation, particularly in systemic disorders where functional outcomes diverge from gene expression trends alone.

While omics profiling defines the molecular landscape of barrier integrity, metabolic stress, and immune activation, it does not by itself establish causality or predict intervention efficacy, necessitating complementary hypothesis-driven modeling approaches.

Complementary glycomic profiling offers additional insight into epithelial and vascular barrier status by capturing changes in extracellular matrix composition. Foundational research has demonstrated that heparan sulfate (HS) proteoglycans, present both in the extracellular matrix and on cell surfaces, exhibit organ-specific structural variation in mice (Warda et al., 2006). Such differences in HS distribution and disaccharide composition highlight the importance of glycosaminoglycan remodeling in regulating cell–cell interactions, signaling pathways, and barrier integrity. Within the context of GBI research, these findings

underscore how glycomic heterogeneity may contribute to variability in endothelial and epithelial function, thereby influencing neuroimmune outcomes in laboratory animal models. In multi-barrier GBI research, these glycomic shifts serve as high-resolution markers for altered permeability and immune cell trafficking (Warda et al., 2008), providing a refined context for interpreting experimental phenotypes. Together, proteo-glycomic signatures provide a refined molecular context for interpreting experimental phenotypes and validating that laboratory models reflect integrated physiological processes. When viewed as an integrated pipeline rather than isolated techniques, omics profiling, computational modeling, and in vitro testing collectively function as a methodological continuum that filters biological noise and refines experimental intent.

In Silico and In Vitro Validation as Gatekeepers Before Animal Testing

Building upon molecular profiling, in silico modeling provides a hypothesis-generating layer that enables targeted exploration of molecular interactions but requires empirical validation before translation to animal studies. Furthermore, in the context of laboratory animal science, the application of in silico approaches including molecular docking, molecular dynamics (MD) simulations, and systems-level computational modeling serves as a vital refinement strategy in accordance with the 3Rs principles. These tools allow for the generation of high-confidence, testable hypotheses regarding the interactions between environmental toxicants, bioactive compounds, and physiological signaling pathways before or alongside in vivo trials.

Recent integrated studies have demonstrated the power of combining computational analysis with empirical laboratory data to dissect complex toxicological and therapeutic mechanisms. For instance, in silico and in vivo dissection has been utilized to evaluate the protective potentials of nano-scale interventions and phytotherapy against chemically-induced systemic toxicities (Ibrahim et al., 2026). Furthermore, molecular docking has proven essential in elucidating the mechanistic pathways through which bioactive compounds, such as P-coumaric acid, offer hepatoprotection against environmental contaminants like bisphenol A (Tekin et al., 2025). These methods are not limited to mammalian models; they also extend to comparative pathology and veterinary science, where combined in silico and in vitro studies have identified the antiparasitic efficacy of botanical extracts (Attia et al., 2025).

Importantly, within a rigorous preclinical validation framework, these in silico outputs are framed as hypothesis-supporting tools that inform downstream experimental prioritization. By identifying specific molecular 'lock-and-key' interactions between ligands and biological targets (Attia et al., 2025; Tekin et al., 2025), researchers can refine their experimental design, thereby reducing the 'experimental noise' and the number of animals required for empirical validation. This integrated approach ensures that laboratory animal practices remain focused on mechanistically plausible targets, directly enhancing the reproducibility and ethical standards of the research (Ibrahim et al., 2026).

Accordingly, in vitro validation serves as the essential intermediary step that tests computational predictions under controlled biological conditions. In vitro validation represents a critical intermediary step between computational prediction and animal experimentation. Assays such as simulated gastric and intestinal fluid exposure, protease challenge studies, and physicochemical stability testing allow investigators to assess formulation robustness under conditions relevant to the rodent gastrointestinal environment (Minekus et al., 2014). Release-kinetics analyses and permeability models, including epithelial monolayer systems, further support evaluation of dose recovery, exposure consistency, and barrier interaction parameters that directly influence interpretability in GBI-focused studies.

Complementary immune cell–based assays provide additional insight into biological plausibility by enabling controlled assessment of inflammatory signaling, cytotoxicity, or immune activation in response to candidate formulations (Wellach & Riemer, 2025). When interpreted cautiously, these systems can help distinguish formulation-driven effects from compound-intrinsic activity and identify conditions under which sterile inflammation or off-target immune activation may distort in vivo outcomes. In this context, human T-cell based models offer a valuable translational reference for probing conserved immune

signaling pathways and inflammasome-associated responses, serving as an upstream screening tool to refine experimental hypotheses and minimize immune-related confounding before animal studies are initiated (Park et al., 2025).

Taken together, omics profiling, in silico modeling, and in vitro validation function as a staged methodological filter that refines experimental intent before animal exposure. This integrated approach reduces variability, supports ethical refinement, and improves interpretability by ensuring that in vivo studies are grounded in biological plausibility and functional stability.

Implications for Experimental Rigor and Translational Integrity

Integrating methodological refinement with staged validation provides a foundation for interpreting in vivo findings within their appropriate physiological and experimental context.

Implications for Experimental Rigor in Laboratory Animal Science

The integration of omics profiling, in silico modeling, and staged in vitro validation strengthens the interpretive framework of laboratory animal research by situating in vivo outcomes within a broader physiological context. Rather than replacing animal experimentation, these approaches refine experimental intent and contextualize animal-derived data within interacting layers of barrier integrity, metabolic state, immune activation, and environmental exposure. When applied systematically, staged validation pipelines provide a practical means to reduce experimental variability, improve reproducibility, and enhance translational relevance across GBI axis research and laboratory animal science more broadly.

One Health Perspective and Translational Integrity

From a One Health perspective, laboratory animal models represent a critical interface between environmental exposures, host biology, and disease mechanisms shared across species. Integrating ethical refinement, stress reduction, biologically justified model selection, and rigorous tissue handling strengthens the translational value of GBI research by ensuring that observed outcomes reflect conserved biological processes such as barrier dysfunction, metabolic stress, and immune activation rather than procedural artifacts (Bailey et al., 2011; Moradian et al., 2024; Park et al., 2025).

By aligning methodological rigor with animal welfare considerations, laboratory animal science advances toward more reproducible, ethically responsible, and biologically meaningful research. This integrated approach reinforces the role of laboratory animals as valid translational models within a broader One Health framework and supports international goals for high-quality biomedical research.

Conclusion and Recommendations

Research on the GBI axis has advanced rapidly; however, its biological complexity necessitates careful integration of mechanistic insight with core principles of laboratory animal science. This review highlights that GBI-related outcomes are shaped not only by intestinal processes but also by coordinated interactions across multiple epithelial barriers, immune priming states, and neurophysiological clearance systems. These mechanisms operate within experimental contexts that are highly sensitive to species susceptibility, environmental conditions, and methodological choices.

Comparative pathology across wild and laboratory species demonstrates that animals are not biologically neutral experimental platforms but possess inherent predispositions that influence disease expression, immune responsiveness, and barrier integrity. Recognition of species- and strain-specific susceptibility is therefore essential for appropriate model selection, accurate interpretation of outcomes, and translational relevance. Failure to account for such biological context risks misattribution of experimental effects and contributes to variability across studies.

A central conclusion of this review is that several sources of experimental bias remain underappreciated in laboratory animal research, including housing- and stress-related variables, tissue collection practices, residual intravascular blood, and perimortem neuroendocrine responses. These factors can significantly influence inflammatory, metabolic, and neuroimmune readouts, particularly in highly vascularized organs and stress-sensitive pathways. Addressing these confounders through refined

experimental design and transparent reporting is critical for improving reproducibility and reducing unnecessary animal use. Emerging methodological approaches, including nano-enabled delivery platforms are discussed as experimental tools to improve dosing consistency and mechanistic interpretability, with the potential to reduce animal use through enhanced reproducibility and refinement.

Ultimately, progress in GBI-axis research depends on aligning mechanistic innovation with rigorous laboratory animal science. Embedding ethical refinement, stress reduction, biologically justified model selection, and methodological transparency within a One Health framework will strengthen scientific robustness while supporting animal welfare and translational integrity.

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References

Ardicli, S., Ardicli, O., Yazici, D., Pat, Y., Babayev, H., Xiong, P., Zeyneloglu, C., Garcia-Sanchez, A., Shi, L. L., Viscardi, O. G., Skolnick, S., Ogulur, I., Dhir, R., Jutel, M., Agache, I., Janda, J., Pali-Schöll, I., Nadeau, K. C., Akdis, M., & Akdis, C. A. (2024). Epithelial barrier dysfunction and associated diseases in companion animals: Differences and similarities between humans and animals and research needs. *Allergy*, *79*(12), 3238–3268. https://doi.org/10.1111/all.16343

Asimakidou, E., Saipuljumri, E. N., Lo, C. H., & Zeng, J. (2025). Role of metabolic dysfunction and inflammation along the liver-brain axis in animal models with obesity-induced neurodegeneration. *Neural Regeneration Research*, *20*(4), 1069–1076. https://doi.org/10.4103/NRR.NRR-D-23-01770

Attia, M. M., Abdelsalam, M., Mohamed, H. I., Shadidizaji, A., Soliman, A. W., & Warda, M. (2025). *Punica granatum* extract demonstrates antiparasitic effects against *Caligus clemensi* through in silico and in vitro studies. *Scientific Reports 15(*1):35005. https://doi.org/10.1038/s41598-025-19529-y

Bailey, M. T., Dowd, S. E., Galley, J. D., Hufnagle, A. R., Allen, R. G., & Lyte, M. (2011). Exposure to a social stressor alters the structure of the intestinal microbiota: implications for stressor-induced immunomodulation. *Brain, Behavior, and Immunity*, *25*(3), 397–407. https://doi.org/10.1016/j.bbi.2010.10.023

Banerjee, A., Goswami, A., & Choudhury, A. (2026). Convergence in nanomedicine: integrating brain-targeted delivery and gut microbiota modulation for neurological protection. *BioNanoScience 16*(1), 75. https://doi.org/10.1007/s12668-025-02276-9

Bohr, T., Hjorth, P. G., Holst, S. C., Hrabětová, S., Kiviniemi, V., Lilius, T., Lundgaard, I., Mardal, K. A., Martens, E. A., Mori, Y., Nägerl, U. V., Nicholson, C., Tannenbaum, A., Thomas, J. H., Tithof, J., Benveniste, H., Iliff, J. J., Kelley, D. H., & Nedergaard, M. (2022). The glymphatic system: Current understanding and modeling. *iScience, 25*(9), 104987. https://doi.org/10.1016/j.isci.2022.104987

Chen, B., Meseguer, D., Lenck, S., Thomas, J. L., & Schneeberger, M. (2025). Rewiring of the glymphatic landscape in metabolic disorders. *Trends in Endocrinology and Metabolism: TEM, 36*(8), 710–720. https://doi.org/10.1016/j.tem.2024.11.005

Chen, S., Wang, H., Zhang, L., Xi, Y., Lu, Y., Yu, K., Zhu, Y., Regina, I., Bi, Y., & Tong, F. (2025). Glymphatic system: a self-purification circulation in brain. *Frontiers in Cellular Neuroscience, 19,* 1528995. https://doi.org/10.3389/fncel.2025.1528995

Decout, A., Katz, J. D., Venkatraman, S., & Ablasser, A. (2021). The cGAS-STING pathway as a therapeutic target in inflammatory diseases. *Nature Reviews. Immunology, 21*(9), 548–569. https://doi.org/10.1038/s41577-021-00524-z

Devan, S. R. K., Ingle, A., Ramachandra, S. G., Hegde, N., Ramesh, S., Nagarajan, P., Muthukumar, S. P., Buragohain, R., Singh, V. P., & Tamhankar, C. (2024). Advancements of 3Rs in biomedical research, laboratory animal science and welfare: International perspectives. *Journal of Laboratory Animal Science, 7*(2), 74–96. https://doi.org/10.48165/jlas.2024.7.2.6

Dinan, T. G., & Cryan, J. F. (2017). The microbiome-gut-brain axis in health and disease. *Gastroenterology Clinics of North America*, *46*(1), 77–89. https://doi.org/10.1016/j.gtc.2016.09.007

Ding, S. B., Chu, X. L., Jin, Y. X., Jiang, J. J., Zhao, X., & Yu, M. (2023). Epigallocatechin gallate alleviates high-fat diet-induced hepatic lipotoxicity by targeting mitochondrial ROS-mediated ferroptosis. *Frontiers in Pharmacology, 14,* 1148814. https://doi.org/10.3389/fphar.2023.1148814

Elshareif, N., Gornick, E., Gavini, C. K., Aubert, G., & Mansuy-Aubert, V. (2023). Comparison of western diet-induced obesity and streptozotocin mouse models: insights into energy balance, somatosensory dysfunction, and cardiac autonomic neuropathy. *Frontiers in Physiology, 14*, 1238120. https://doi.org/10.3389/fphys.2023.1238120

Gakuba, C., Gaberel, T., Goursaud, S., Bourges, J., Di Palma, C., Quenault, A., Martinez de Lizarrondo, S., Vivien, D., & Gauberti, M. (2018). General anesthesia inhibits the activity of the "glymphatic system". *Theranostics, 8*(3), 710–722. https://doi.org/10.7150/thno.19154

Groll, T., Aupperle-Lellbach, H., Mogler, C., & Steiger, K. (2024). Vergleichende pathologie in der onkologischen forschung. *Die Pathologie, 4*, 190-197.

https://doi.org/10.1007/s00292-024-01327-4
Guan, X., Li, H., Zhang, L., & Zhi, H. (2025). Mechanisms of mitochondrial damage-associated molecular patterns associated with inflammatory response in cardiovascular diseases. *Inflammation Research, 74*(1), 18. https://doi.org/10.1007/s00011-025-01993-x
Hauglund, N. L., & Nedergaard, M. (2025). Is glymphatic clearance the secret to restorative sleep?. *Brain : a Journal of Neurology*, awaf453. Advance online publication. https://doi.org/10.1093/brain/awaf453
Hu, Q., Li, C., Zhang, T., Yi, L., Shan, Y., Ma, X., Cai, T., Ran, L., Shen, H., & Li, Y. (2024). Dihydromyricetin suppresses endothelial NLRP3 inflammasome activation and attenuates atherogenesis by promoting mitophagy. *Lipids in Health and Disease*, *23*(1), 279. https://doi.org/10.1186/s12944-024-02263-1
Ibrahim, M. A., Aboukhezam, B., Aboubakr, F. A., Yousef, E. M., Saihati, H. A. A., Nasr, W. S. S. E., Shadidizaji, A., Algohary, A. M., Ahmed Farid, O. A., & Warda, M. (2026). In vivo and in silico dissection of triclosan-induced reproductive toxicity: protective potentials of nanoselenium and phytotherapy. *Basic & Clinical Pharmacology & Toxicology, 138*(1), e70161. https://doi.org/10.1111/bcpt.70161
Iliff, J. J., Wang, M., Liao, Y., Plogg, B. A., Peng, W., Gundersen, G. A., Benveniste, H., Vates, G. E., Deane, R., Goldman, S. A., Nagelhus, E. A., & Nedergaard, M. (2012). A paravascular pathway facilitates CSF flow through the brain parenchyma and the clearance of interstitial solutes, including amyloid β. *Science Translational Medicine*, *4*(147), 147ra111. https://doi.org/10.1126/scitranslmed.3003748
Kamble, M.G., Singh, A., Singh, S.V., Kamble, M. G., Sagar, N. A., & Rani, Nitu. (2025). Nanotechnology for encapsulation of bioactive components: a review. *Discover Food, 5,* 116. https://doi.org/10.1007/s44187-025-00386-7
Kim, M. E., Lim, Y., & Lee, J. S. (2025). Mitochondrial dysfunction and metabolic reprogramming in chronic inflammatory diseases: molecular insights and therapeutic opportunities. *Current Issues in Molecular Biology*, *47*(12), 1042. https://doi.org/10.3390/cimb47121042
Kim, N., Kim, H., Youm, J. B., Park, W. S., Warda, M., Ko, J. H., & Han, J. (2006). Site specific differential activation of ras/raf/ERK signaling in rabbit isoproterenol-induced left ventricular hypertrophy. *Biochimica et Biophysica Acta*, *1763*(10), 1067–1075. https://doi.org/10.1016/j.bbamcr.2006.08.002
Konopko, A., Kazek, M., Waraksa-Zasada, E., Łukomska, A., Ratajczak, J., Kucia, M., & Ratajczak, M. Z. (2025). The NLRP3 inflammasome transmits sterile inflammation signals to sustain proper mitochondrial electron transport chain function and influences cellular metabolism. *Stem Cell Reviews and Reports*, *21*(7), 2157–2169. https://doi.org/10.1007/s12015-025-10948-y
Krings, T., Takemoto, Y., Mori, K., & Kee, T. P. (2025). The glymphatic system and its role in neurovascular diseases. *Journal of Neuroendovascular Therapy*, *19*(1), 2025-0020. https://doi.org/10.5797/jnet.ra.2025-0020
Lange, S., & Inal, J. M. (2024). Animal models of human disease 2.0. *International Journal of Molecular Sciences*, *25*(24), 13743. https://doi.org/10.3390/ijms252413743
Le Maho, Y., Tasiemski, A., Bertile, F., Bulet, P. (2025). Fieldwork on animals living in extreme conditions as a source of biomedical innovation. Science in One Health, 4, 100096. https://doi.org/10.1016/j.soh.2024.100096
Lee, H., Xie, L., Yu, M., Kang, H., Feng, T., Deane, R., Logan, J., Nedergaard, M., & Benveniste, H. (2015). The effect of body posture on brain glymphatic transport. *The Journal of Neuroscience, 35*(31), 11034–11044. https://doi.org/10.1523/JNEUROSCI.1625-15.2015
Liu, Y., Beyer, A., & Aebersold, R. (2016). On the dependency of cellular protein levels on mrna abundance. *Cell, 165*(3), 535–550. https://doi.org/10.1016/j.cell.2016.03.014
Lopes, D. M., Wells, J. A., Ma, D., Wallis, L., Park, D., Llewellyn, S. K., Ahmed, Z., Lythgoe, M. F., & Harrison, I. F. (2024). Glymphatic inhibition exacerbates tau propagation in an Alzheimer's disease model. *Alzheimer's Research & Therapy*, *16*(1), 71. https://doi.org/10.1186/s13195-024-01439-2
Mangiaterra, S., Marker, L., Cerquetella, M., Galosi, L., Marchegiani, A., Gavazza, A., & Rossi, G. (2022). Chronic stress-related gastroenteric pathology in cheetah: relation between intrinsic and extrinsic factors. *Biology*, *11*(4), 606. https://doi.org/10.3390/biology11040606
Marei H. E. (2025). Epigenetic editing in neurological and neuropsychiatric disorders: Pioneering next-gen therapeutics for precision gene control. *Molecular Neurobiology*, *63*(1), 330. https://doi.org/10.1007/s12035-025-05590-1
Martini E. (2025). Norepinephrine oscillations regulate glymphatic clearance during spleen. *Nature Cardiovascular Research*, *4*(2), 121. https://doi.org/10.1038/s44161-025-00616-2
McGill J. L. (2025). Beyond the mouse: nontraditional animal models in immunology. *Journal of Immunology*, *214*(10), 2492–2493. https://doi.org/10.1093/jimmun/vkaf238
Minekus, M., Alminger, M., Alvito, P., Ballance, S., Bohn, T., Bourlieu, C., Carrière, F., Boutrou, R., Corredig, M., Dupont, D., Dufour, C., Egger, L., Golding, M., Karakaya, S., Kirkhus, B., Le Feunteun, S., Lesmes, U., Macierzanka, A., Mackie, A., Marze, S., … Brodkorb, A. (2014). A standardised static in vitro digestion method suitable for food - an international consensus. *Food & Function*, *5*(6), 1113–1124. https://doi.org/10.1039/c3fo60702j
Missal, P., Verma, S., Singh, A., & Kar, S. K. (2024). Gut-brain axis: A medium for immune interaction. In N. Rezaei & N. Yazdanpanah (Eds.), *PsychoNeuroImmunology* (Vol. 30 pp. 621-655). Springer. https://doi.org/10.1007/978-3-031-73061-0_19
Mogensen, F. L., Delle, C., & Nedergaard, M. (2021). The glymphatic system (en)during inflammation. *International Journal of Molecular Sciences*, *22*(14), 7491. https://doi.org/10.3390/ijms22147491
Moradian, H., Gabriel, T., Barrau, M., Roblin, X., & Paul, S. (2024). New methods to unveil host-microbe interaction mechanisms along the microbiota-gut-brain-axis. *Gut Microbes*, *16*(1), 2351520.

https://doi.org/10.1080/19490976.2024.2351520
Morys, J., Małecki, A., & Nowacka-Chmielewska, M. (2024). Stress and the gut-brain axis: an inflammatory perspective. *Frontiers in Molecular Neuroscience*, *17*, 1415567. https://doi.org/10.3389/fnmol.2024.1415567
Mukherjee, P., Roy, S., Ghosh, D., & Nandi, S. K. (2022). Role of animal models in biomedical research: a review. *Laboratory Animal Research*, *38*(1), 18. https://doi.org/10.1186/s42826-022-00128-1
Müller, L., & Di Benedetto, S. (2025). Bridging the brain and gut: neuroimmune mechanisms of neuroinflammation and therapeutic insights. *Frontiers in Cellular Neuroscience*, *19*, 1590002. https://doi.org/10.3389/fncel.2025.1590002
National Research Council (US) Institute for Laboratory Animal Research. 1996. *Guide for the Care and Use of Laboratory Animals*. Washington (DC): National Academies Press (US).
Okumura, R., & Takeda, K. (2024). The role of the mucosal barrier system in maintaining gut symbiosis to prevent intestinal inflammation. *Seminars in Immunopathology*, *47*(1), 2. https://doi.org/10.1007/s00281-024-01026-5
O'Riordan, K. J., Moloney, G. M., Keane, L., Clarke, G., & Cryan, J. F. (2025). The gut microbiota-immune-brain axis: Therapeutic implications. *Cell Reports Medicine*, *6*(3), 101982. https://doi.org/10.1016/j.xcrm.2025.101982
Park, J. C., Chang, L., Kwon, H. K., & Im, S. H. (2025). Beyond the gut: decoding the gut-immune-brain axis in health and disease. *Cellular & Molecular Immunology*, *22*(11), 1287–1312. https://doi.org/10.1038/s41423-025-01333-3
Pereira, H., Hoffman, J. I., Krüger, O., Czirják, G. Á., Rinaud, T., Ottensmann, M., Gladow, K. P., Caspers, B. A., Maraci, Ö., Kaiser, S., & Chakarov, N. (2024). The gut microbiota-immune-brain axis in a wild vertebrate: dynamic interactions and health impacts. *Frontiers in Microbiology*, *15*, 1413976. https://doi.org/10.3389/fmicb.2024.1413976
Pinzón-Fernández, M. V., Saavedra-Torres, J. S., López Garzón, N. A., Pachon-Bueno, J. S., Tamayo-Giraldo, F. J., Rojas Gomez, M. C., Arias-Intriago, M., Gaibor-Pazmiño, A., López-Cortés, A., & Izquierdo-Condoy, J. S. (2025). NLRP3 and beyond: inflammasomes as central cellular hub and emerging therapeutic target in inflammation and disease. *Frontiers in Immunology*, *16*, 1624770. https://doi.org/10.3389/fimmu.2025.1624770
Rothenburger, J. L., Himsworth, C. G., Clifford, C. B., Ellis, J., Treuting, P. M., & Leighton, F. A. (2015). Respiratory pathology and pathogens in wild urban rats (Rattus norvegicus and Rattus rattus). *Veterinary pathology*, *52*(6), 1210–1219. https://doi.org/10.1177/0300985815593123
Saihati, H. A. A., Ahmed, B. Y., Mosaad, R. M., El-Garhy, H. A. S., Bakeer, R. M., Yousef, E. M., Ahmed, I. M., Nasr, W. S. S. E., Shadi-Dizaji, A., Ahmed-Farid, O. A., & Warda, M. (2026). Signal-level determinants of cognitive decline with PPIs versus H_2RAs: Transportome (CBLIF/TCN2) and CHRNA7 nodes. *Molecular Nutrition & Food Research*, *70*(3), e70382. https://doi.org/10.1002/mnfr.70382
Sajjanar, B., Krishnaswamy, N., Saxena, V. K., & Dhara, S. K. (2025). Stress responses to changing environmental factors in the domestic animals: An epigenetic perspective. *Journal of Animal Physiology and Animal Nutrition*, *109*(4), 1000–1016. https://doi.org/10.1111/jpn.14115
Sarkar, A., Lehto, S. M., Harty, S., Dinan, T. G., Cryan, J. F., & Burnet, P. W. J. (2016). Psychobiotics and the manipulation of bacteria-gut-brain signals. *Trends in Neurosciences*, *39*(11), 763–781. https://doi.org/10.1016/j.tins.2016.09.002
Sato, H., Yamada, K., Miyake, M., & Onoue, S. (2023). Recent advancements in the development of nanocarriers for mucosal drug delivery systems to control oral absorption. *Pharmaceutics*, *15*(12), 2708. https://doi.org/10.3390/pharmaceutics15122708
Sikes, R. S., & Paul, E. (2013). Fundamental differences between wildlife and biomedical research. *ILAR journal*, *54*(1), 5–13. https://doi.org/10.1093/ilar/ilt015
Skinner M. K. (2025). Environmental Epigenetics 2025 update. *Environmental Epigenetics*, *11*(1), dvaf004. https://doi.org/10.1093/eep/dvaf004
Singh, R., & Lillard, J. W., Jr (2009). Nanoparticle-based targeted drug delivery. *Experimental and Molecular Pathology*, *86*(3), 215–223. https://doi.org/10.1016/j.yexmp.2008.12.004
Stolfi, C., Maresca, C., Monteleone, G., & Laudisi, F. (2022). Implication of intestinal barrier dysfunction in gut dysbiosis and diseases. *Biomedicines*, *10*(2), 289. https://doi.org/10.3390/biomedicines10020289
Suárez-Bonnet, A., & Ramírez Rivero, G. A. (2023). Veterinary comparative pathology, a scientific tool for a thriving planet. *Animals*, *13*(9), 1504. https://doi.org/10.3390/ani13091504
Sun, S., Han, Y., Li, H., Wang, C., Zhou, S., Zhang, X., Dai, S., Peng, Y., & Wang, Z. (2025). Beyond the genome: epigenetic regulation of immune responses and T cells in brain tumors. *Frontiers in Immunology*, *16*, 1690552. https://doi.org/10.3389/fimmu.2025.1690552
Swanson, K. V., Deng, M., & Ting, J. P. (2019). The NLRP3 inflammasome: molecular activation and regulation to therapeutics. *Nature reviews. Immunology*, *19*(8), 477–489. https://doi.org/10.1038/s41577-019-0165-0
Tamam, O. A. S. (2014). Parasitic perifollicular dermatitis in the egyptian lesser blind mole rat (spalax leucodon egyptiacus). *Bangladesh Journal of Veterinary Medicine, 12*(2), 197–201. https://doi.org/10.3329/bjvm.v12i2.21291
Tekin, S., Bolat, M., Atasever, A., Bolat, İ., Çinar, B., Shadidizaji, A., Dağ, Y., Şengül, E., Yildirim, S., Hacimuftuoglu, A., & Warda, M. (2025). Mechanistic insights into the P-coumaric acid protection against bisphenol A-induced hepatotoxicity in in vivo and in silico models. *Scientific Reports*, *15*(1), 11023. https://doi.org/10.1038/s41598-025-87099-0
Terio, K. A., Munson, L., Marker, L., Aldridge, B. M., & Solnick, J. V. (2005). Comparison of helicobacter spp. in cheetahs (Acinonyx jubatus) with and without gastritis. *Journal of Clinical Microbiology*, *43*(1), 229–234. https://doi.org/10.1128/JCM.43.1.229-234.2005
Tordiffe, A. S. W. (2017). *The metabolic profiling of cheetahs*

(acinonyx jubatus): A systems biology approach to understanding the chronic diseases they suffer in captivity. [Doctoral Thesis, North-West University].

Vasciaveo, V., Iadarola, A., Casile, A., Dante, D., Morello, G., Minotta, L., Tamagno, E., Cicolin, A., & Guglielmotto, M. (2023). Sleep fragmentation affects glymphatic system through the different expression of AQP4 in wild type and 5xFAD mouse models. *Acta Neuropathologica Communications*, *11*(1), 16. https://doi.org/10.1186/s40478-022-01498-2

Vijayaram, S., Mahendran, K., Razafindralambo, H., Ringø, E., Kannan, S., & Sun, Y. Z. (2025). Probiotics, gut microbiota, and brain health: Exploring therapeutic pathways. *AIMS Microbiology*, *11*(3), 501–541. https://doi.org/10.3934/microbiol.2025022

Wang, D. J., Hua, J., Cao, D., & Ho, M. L. (2023). Neurofluids and the glymphatic system: anatomy, physiology, and imaging. *The British Journal of Radiology*, *96*(1151), 20230016. https://doi.org/10.1259/bjr.20230016

Wang, H., Ayala, A., Aziz, M., Billiar, T. R., Deutschman, C. S., Jeyaseelan, S., Tang, D., & Wang, P. (2025). Value of animal sepsis research in navigating the translational labyrinth. *Frontiers in Immunology*, *16*, 1593342. https://doi.org/10.3389/fimmu.2025.1593342

Warda, M., & Zeisig, R. (2000). Phospholipid- and fatty acid-composition in the erythrocyte membrane of the one-humped camel [Camelus dromedarius] and its influence on vesicle properties prepared from these lipids. *DTW. Deutsche tierarztliche Wochenschrift*, *107*(9), 368–373.

Warda, M., Tekin, S., Gamal, M., Khafaga, N., Çelebi, F., & Tarantino, G. (2025). Lipid rafts: novel therapeutic targets for metabolic, neurodegenerative, oncological, and cardiovascular diseases. *Lipids in Health and Disease*, *24*(1), 147. https://doi.org/10.1186/s12944-025-02563-0

Warda, M., Toida, T., Zhang, F., Sun, P., Munoz, E., Xie, J., & Linhardt, R. J. (2006). Isolation and characterization of heparan sulfate from various murine tissues. *Glycoconjugate Journal*, *23*(7-8), 555–563. https://doi.org/10.1007/s10719-006-7668-1

Warda, M., Zhang, F., Radwan, M., Zhang, Z., Kim, N., Kim, Y. N., Linhardt, R. J., & Han, J. (2008). Is human placenta proteoglycan remodeling involved in pre-eclampsia?. *Glycoconjugate Journal*, *25*(5), 441–450. https://doi.org/10.1007/s10719-007-9090-8

Weber, A. N. R., McManus, R. M., Hornung, V., Geyer, M., Kuemmerle-Deschner, J. B., & Latz, E. (2025). The expanding role of the NLRP3 inflammasome from periodic fevers to therapeutic targets. *Nature Immunology*, *26*(9), 1453–1466. https://doi.org/10.1038/s41590-025-02230-7

Wellach, K., & Riemer, A. B. (2025). Highly sensitive live-cell imaging-based cytotoxicity assay enables functional validation of rare epitope-specific CTLs. *Frontiers in Immunology*, *16*, 1558620. https://doi.org/10.3389/fimmu.2025.1558620

Wiriansya, E. P., Rahman, D., Zuhair, M. N., Rijal, S., Ikram, D., & Pangnguriseng, U. A. (2023). Effects of e-cigarette vapor smoke on pulmonary alveoli in rattus norvegicus lungs. *Journal of Respirology 9*(3), 200–205. https://doi.org/10.20473/jr.v9-I.3.2023.200-205

Wu, M., Yu, C., Wen, F., Li, Y., Zhang, X., Wang, Y., Chen, X., &Chen, X. (2025). NLRP3 inflammasome inhibits mitophagy during the progression of temporal lobe epilepsy. *Scientific Reports, 15*(1), 16341. https://doi.org/10.1038/s41598-025-01087-y

Xie, L., Kang, H., Xu, Q., Chen, M. J., Liao, Y., Thiyagarajan, M., O'Donnell, J., Christensen, D. J., Nicholson, C., Iliff, J. J., Takano, T., Deane, R., & Nedergaard, M. (2013). Sleep drives metabolite clearance from the adult brain. *Science*, *342*(6156), 373–377. https://doi.org/10.1126/science.1241224

Xu, Y., Yin, H., Li, L., Wang, X., & Hou, Q. (2025). Covert cerebrospinal fluid dynamics dysfunction: evolution from conventional to innovative therapies. *Frontiers in Neurology*, *16*, 1554813. https://doi.org/10.3389/fneur.2025.1554813