Human-Relevant Non-Animal Methodologies (NAMs)
Specialised Models Series: **Neurological & Psychiatric**

Neurodegenerative Disease Models
Alzheimers, Parkinsons, Huntingdon, ALS

Reference 3.3 in Full Scope Roadmap

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Legal & Scientific Imperative for Universities

Section 2A of the *Animals (Scientific Procedures) Act 1986* imposes a statutory duty on the Secretary of State to ensure compliance with the principles of replacement, reduction, and refinement. The principle of replacement requires that, wherever a scientifically satisfactory method or testing strategy not entailing the use of protected animals is available, it must be used to replace the use of a regulated procedure.

Traditional drug discovery pipelines for neurodegenerative diseases (AD, PD, HD, and ALS) rely heavily on transgenic rodent lines designed to overexpress mutant human proteins. These platforms suffer from severe translational limitations. These limitations are evident in the following critical disparities:

Failure to Recapitulate Pathophysiology: Animal models fail to replicate key hallmarks of human disease, such as selective neuronal vulnerability, spontaneous progressive cell death, and human-specific glial-neuronal inflammatory dynamics.

Protein Sequence & Splice Variant Mismatch: Human-restricted pathologies—including hyperphosphorylated tau, specific amyloid-beta oligomers, alpha-synuclein aggregates, toxic TDP-43 mislocalisation, and mutant Huntingtin (mHTT) configurations—cannot be accurately modelled in animals.

Clinical Translational Failure: Due to these fundamental species differences, the translational failure rate for neurodegenerative drugs remains close to 100%.

Ethical Severity of Models: Legacy models often involve progressive neurological impairment and motor decline, providing a significant ethical and regulatory mandate to transition away from these regulated procedures.

Transitioning to human-relevant Non-Animal Methodologies (NAMs)—such as patient-derived iPSC brain organoids, microfluidic models of protein aggregation, and predictive computational modelling—is both a scientific necessity to unlock successful clinical pathways and a legal mandate for UK universities under ASPA 1986.

Landmark Regulatory & Scientific Momentum

Significant progress has been made in the development and application of human-relevant neurodegenerative NAMs:

- **Midbrain and Cortical Organoids:** Recreate human midbrain architecture containing functional dopaminergic neurons, mimicking Parkinson's Lewy body pathology, and cortical layering to model familial and sporadic Alzheimer's disease.
- **Neuromuscular Junction (NMJ)-on-Chip:** Integrates human iPSC-derived spinal motor neurons and skeletal muscle fibers under physiological microfluidic flow to model motor pathway degradation in ALS.
- **In Silico Protein Aggregation Kinetic Simulators:** Leverage molecular dynamics and structural modelling to predict the nucleation, propagation, and target-binding profiles of toxic human protein aggregates.

Core Specialised Neurodegenerative Platforms

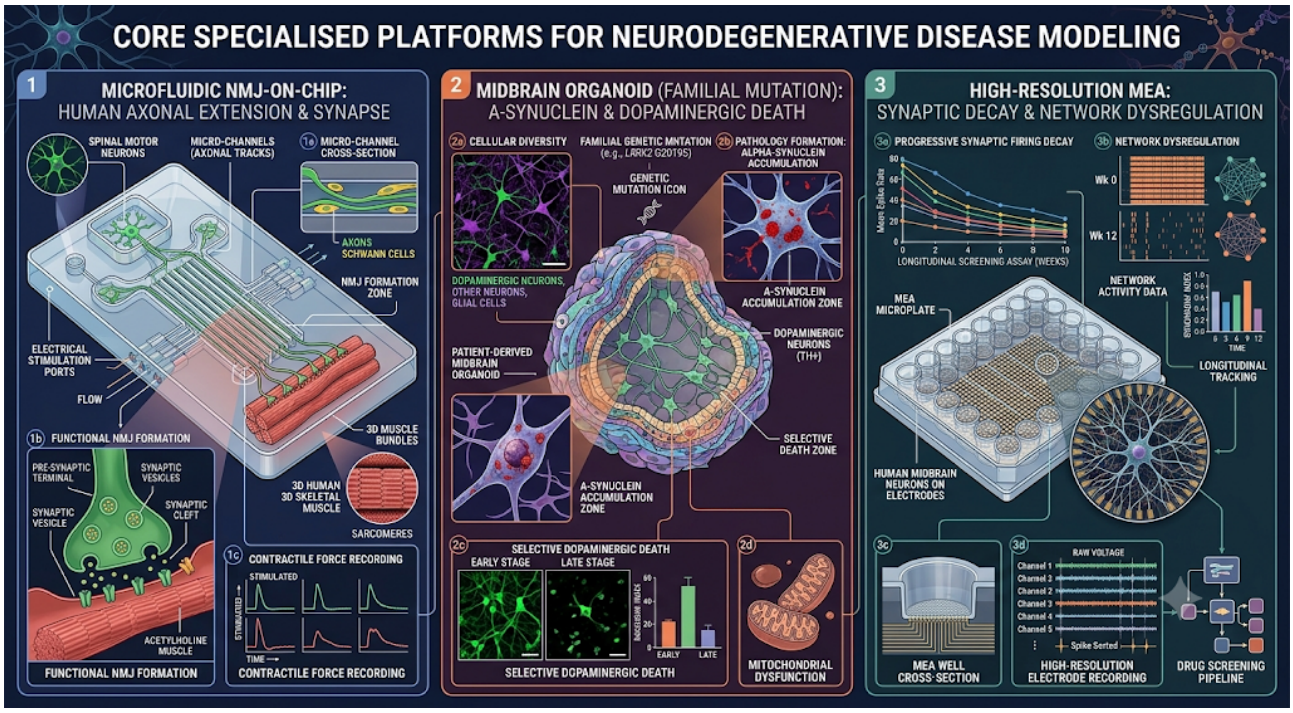

Figure 1: Core Specialised Neurodegenerative Platforms — Microfluidic neuromuscular junction (NMJ-on-Chip) platform showing human spinal motor neurons extending functional axons through micro-channels to synapse with 3D human skeletal muscle bundles *(left)*; 3D patient-derived midbrain organoid harbouring familial genetic mutations, displaying alpha-synuclein accumulation and selective dopaminergic neuronal death *(centre)*; and a high-resolution microelectrode array (MEA) substrate detecting progressive synaptic firing decay and network dysregulation over a longitudinal screening assay *(right)*.

Table 1: Specialised Biological & Tissue-Engineered (In Vitro) Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
iPSC-Derived Midbrain & Cortical Organoids	Self-organizing 3D human brain tissue containing dopaminergic, glutamatergic, and GABAergic neurons co-cultured with astrocytes and microglia.	Modeling progressive protein aggregation (alpha-synuclein, tau, amyloid-beta), selective cell loss, and local neuroinflammation.	Replaces transgenic rodent breeding and aging pipelines that fail to match human-restricted aggregate propagation or glial activation.
Neuromuscular Junction (NMJ)-on-Chip	Microfluidic compartmentalised co-culture systems linking human motor neurons to functional skeletal muscle fibers.	Assessing motor neuron degeneration, axonal transport defects, TDP-43 aggregation, and synaptic degradation in ALS and spinal muscular atrophy.	Eliminates invasive rodent motor function assays (e.g., rotarod, grip-strength tests) and terminal tissue collection.
Assembled Multi-Regional Degeneration Models	Fused regional organoids (e.g., striatal-cortical assembloids) mapping target projection tracts.	Tracking mutant Huntingtin (mHTT) propagation, corticostriatal pathway disconnect, and selective medium spiny neuron vulnerability in Huntington's.	Replaces complex intracranial surgeries, lesion models, and chemical stereotaxic injections in primates and rodents.
High-Throughput Microfluidic Neuro-Glial Arrays	Microfluidic chambers separating neuronal cell bodies from axons, populated with primary or iPSC-derived astrocytes and microglia.	High-content screening of neuroprotective compounds, real-time axonal transport imaging, and neuroimmune-mediated synaptic pruning assays.	Bypasses traditional rodent spinal cord and brain harvesting protocols, shifting early screening into automated cell-based pipelines.

Cross-Cutting & Enabling Platforms for Neurodegenerative Research

These platforms support multiple disease-specific research areas and are widely applicable across small-molecule design, genetic target validation, and pharmacokinetic profiling. While tissue-specific organoids focus on structural niches, these cross-cutting technologies provide the quantitative pipelines required to validate high-maturity NAMs for regulatory-accepted, "fit-for-purpose" research.

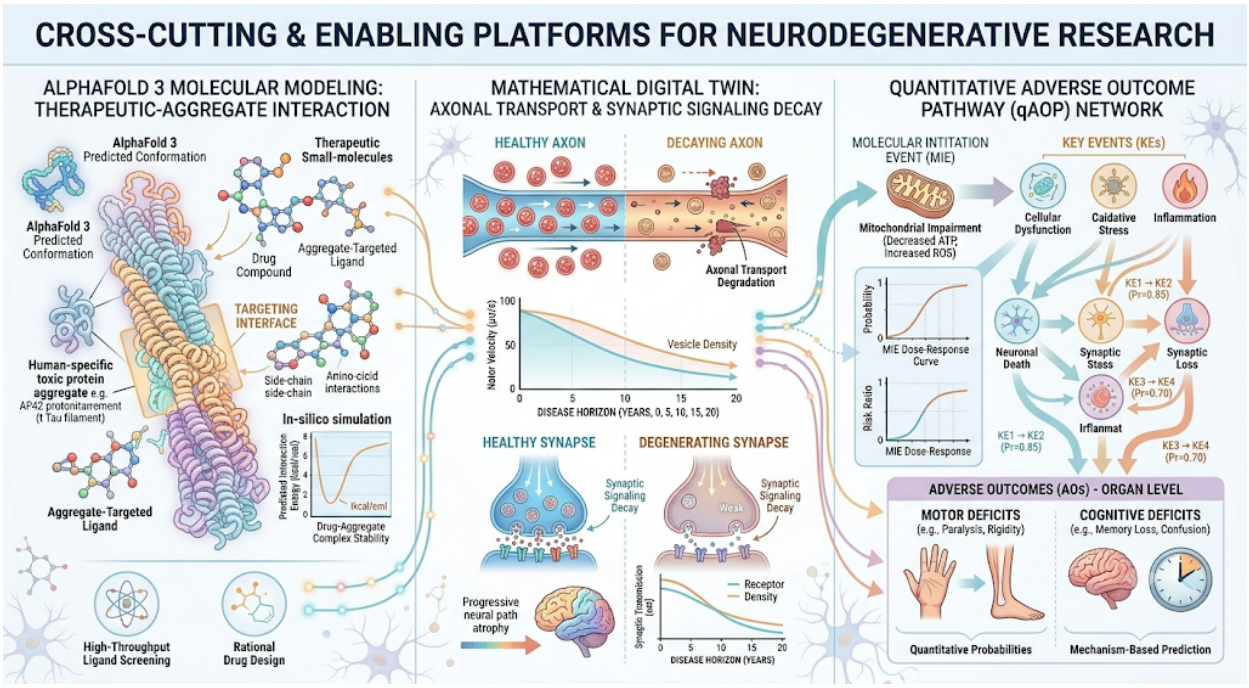

Figure 2: Cross-Cutting & Enabling Platforms for Neurodegenerative Research — AlphaFold 3 molecular modelling projecting the precise conformational interaction of therapeutic small-molecules with human-specific toxic protein aggregates *(left)*; mathematical digital twin simulating multi-scale axonal transport decay and synaptic signalling degradation over longitudinal disease horizons *(centre)*; and a quantitative Adverse Outcome Pathway (qAOP) network mapping molecular initiation events (e.g., mitochondrial impairment) to organ-level motor and cognitive deficits *(right)*.

Table 2: Genetic, Computational & Hybrid Platforms

Platform / Model	Description	Key Applications	Benefits for Universities & 3Rs
AlphaFold & Molecular Dynamics Simulators	Advanced AI-driven structural predictions mapping the interactions of small molecules with misfolded human protein configurations.	Designing aggregate-disrupting therapeutics, profiling target configurations, and predicting blood-brain barrier transport characteristics.	Bypasses the need for animal immunization, serum harvesting, and raw computational validation using animal-derived materials.
Multi-Scale Digital Twins of Synaptic Decay	Kinetic and mathematical computational models representing neuronal energy deficits, mitochondrial dysfunction, and network-level decay.	Simulating long-term drug effects, predicting therapeutic window viability, and designing optimal human clinical trial parameters.	A system-wide in silico framework that replaces longitudinal non-human primate and canine cognitive-decline studies.
qAOP-Integrated Screening Networks	Quantitative Adverse Outcome Pathways linking molecular initiation events (e.g., TDP-43 mutation) to systemic motor and neural failure.	Regulatory safety assessment, environmental neurotoxicity profiling, and high-throughput target prioritisation.	Synthesizes multiple in vitro datasets to provide legally defensible Replacement evidence for Project Licence applications.

Regulatory Software & Strategy Frameworks

Regulatory acceptance of NAMs—referred to as "fit-for-purpose qualification"—is not a one-size-fits-all process. Researchers should utilise the following frameworks to ensure their computational and biological strategies align with the *Animals (Scientific Procedures) Act 1986* and industry best practices:

- **Context of Use (COU) Documentation:** Before deploying models, clearly define the specific scientific question the model is intended to address. This is critical for demonstrating that the NAM is reliable and suitable for its intended regulatory purpose.
- **Technical Characterisation:** Maintain comprehensive Standard Operating Procedures (SOPs) and study plans for both the biological NAM and any software used to analyse its output. This transparency is vital for successful project licence applications.
- **Early Regulatory Engagement:** Leverage institutional or external regulatory support to track "fit-for-purpose" milestones. Early engagement with the Home Office/ASRU is strongly encouraged to test the acceptability of a computational or NAM-based approach before full project submission.
- **Weight-of-Evidence (WoE) Frameworks:** Use integrated digital dashboards to aggregate data from both *in vitro* (NAM) and *in silico* (computational) sources. Building a strong "weight-of-evidence" argument is the most effective way to strengthen Replacement justifications when moving away from traditional animal models.

Skills, Training, and Capacity Building

Successful adoption of high-maturity Non-Animal Methodologies (NAMs) requires a strategic shift in workforce development. To ensure the long-term success of the platforms outlined in this series, universities should prioritise the following training initiatives to build institutional capacity:

- **Integrated Neurobiology & Microengineering:** Training programmes should bridge the gap between bench-top cellular biology, fluidic microengineering, and complex electrophysiology data analysis. Researchers require dual proficiency in maintaining long-term fluidic circuit barriers and leveraging the computational pipelines necessary to interpret high-density microelectrode array data.
- **Regulatory Competency:** Staff should receive formal training on "Fit-for-Purpose" qualification and the specific regulatory expectations of the Home Office/ASRU. This includes understanding how to document evidence for the 3Rs within formal project licence applications to ensure they meet legal standards.
- **Technological Literacy:** Skills in microfluidic device troubleshooting, primary stem-cell tissue differentiation, brain organoid maintenance, and AI-driven structural computational modelling are increasingly critical for modern neurobiology research. Universities should facilitate internal workshops or establish external partnerships to provide PhD students and postdoctoral fellows with hands-on experience in these specialised tools.
- **Ethics & Translation:** Education should focus on the scientific imperative of human-relevant research, emphasising why NAMs provide more predictive insights for clinical outcomes than traditional rodent models. This fosters a culture of excellence and ethical research design that is fully aligned with modern international standards.

Table 3: Skills • Training and Capacity Building

Capability Domain	Focus Area	Impact on 3Rs/Replacement
Technical	Advanced iPSC-derived neurodegenerative organoid culture	Direct replacement of transgenic rodent/ primate models
Bioinformatics	Longitudinal human-specific proteomic/ transcriptomic analysis	Bypasses animal-based brain tissue sampling
Regulatory	ASPA 1986 Compliance & AWERB	Facilitates faster approval for non-animal research
Computational	Protein-aggregation & neuro-degeneration modelling	Eliminates invasive animal cognitive/motor impairment studies

Strategic Note: This investment in human expertise is a key component of a robust, future-proof institutional roadmap, demonstrating to regulators that the institution is committed to the safe and effective operation of high-maturity NAMs.

Clinical Translation Case Studies

Case Study 1: Patient-Derived Midbrain Organoids Outperforming Rodent Lewy Body Models (PD)

- **Background:** A therapeutic compound designed to halt alpha-synuclein propagation showed highly promising clearance rates in transgenic mouse models of Parkinson's Disease. However, in Phase II human clinical trials, the compound demonstrated zero efficacy due to structural differences in the human alpha-synuclein target protein configuration that rodent cells could not replicate.

- **The Paradigm Shift:** An academic research group re-evaluated the drug target using patient-derived midbrain organoids. The human-relevant model correctly predicted the clinical failure, demonstrating that the mouse-derived target was structurally non-equivalent to the human aggregate.

- **The Methodology:** Researchers generated 3D midbrain organoids from patient iPSCs harboring a Parkinson's-linked GBA1 mutation. They applied the therapeutic candidate directly to the organoid cultures and quantified cellular uptake, alpha-synuclein clearance, and dopaminergic cell viability using spatial transcriptomics and multi-channel high-content imaging.

- **University & Legal Takeaway:** This study highlights how high-fidelity human organoids can successfully replace rodent efficacy screening, providing solid legal justification to the Home Office under ASPA 1986 that animal testing was non-predictive.

Case Study 2: Human Neuromuscular Junction-on-Chip Validating ALS Lead Compounds

- **Background:** Traditional pre-clinical evaluation of therapeutics for Amyotrophic Lateral Sclerosis (ALS) historically relies on the SOD1 mouse model. These animals undergo severe, progressive muscle paralysis, respiratory failure, and systemic distress before being sacrificed. This model is associated with high animal suffering and has failed to translate to human clinical success.

- **The Paradigm Shift:** A university research team implemented a microfluidic human-relevant Neuromuscular Junction (NMJ)-on-Chip model, completely replacing the need for transgenic rodent survival studies for target validation.

- **The Methodology:** The team co-cultured human iPSC-derived spinal motor neurons harboring TDP-43 mutations with human skeletal muscle fibers. They validated the therapeutic lead by measuring microfluidic axonal transport velocity, synaptic transmission stability, and muscle contraction frequency in response to chemical stimulation, without using live animals.

- **University & Legal Takeaway:** Demonstrates a highly predictive human alternative that completely replaces high-severity animal protocols, aligning perfectly with Home Office expectations for the application of high-maturity Replacement models.

Executive Summary & Compliance Dashboard

Executive Summary

Neurodegenerative disease drug discovery has been historically hindered by a near 100% translational failure rate, largely due to the use of transgenic rodent models that fail to recapitulate human-specific disease hallmarks like selective neuronal vulnerability and progressive cell death. Species-specific differences in protein sequences—such as hyperphosphorylated tau, alpha-synuclein aggregates, and mutant Huntingtin configurations—render legacy animal platforms scientifically inadequate. This roadmap outlines a transition to high-fidelity, human-relevant NAMs (patient-derived iPSC brain organoids, protein-aggregation microfluidics, and predictive computational modelling) to foster a research paradigm that is more accurate, ethically robust, and fully compliant with the Section 2A statutory duty of the *ASPA 1986*.

Compliance Dashboard

Focus Area	Primary Animal Model	NAM Replacement Technology	ASPA 1986 Status
Neurodegeneration	Transgenic rodent models	iPSC organoids & Protein-aggregation MPS	High

See Appendix: Animal-Free Matrix for the long term strategic implementation roadmap

Maturing Matrix

Phase 1 (Exploratory): Audit current animal use in neurodegenerative disease modelling.

Phase 2 (Pilot): Integrate iPSC-derived organoids and protein-aggregation-on-chip protocols into research pipelines.

Phase 3 (Integration): Implement longitudinal multi-omics analysis of human-specific disease progression.

Phase 4 (Standardisation): Replace 100% of high-severity motor and cognitive decline animal models with qualified human-relevant NAMs.

Final Compliance Statement

All proposed models satisfy the Section 2A statutory duty of *ASPA 1986*. By adopting these methodologies, the institution positions itself at the forefront of the global transition toward human-centric, cruelty-free medical science.

Integration Context: Roadmap Conclusion

The modules detailed in this roadmap—from the fundamental neurodegenerative platforms explored in this series to the broader neurobiological and psychiatric frameworks established previously—demonstrate that replacement is an integrated institutional strategy. By mapping these specialised NAM-based disease models into a unified regulatory framework, the institution moves decisively toward a standardised, human-centric research paradigm. This systemic integration is the essential prerequisite for fulfilling the *ASPA 1986* statutory duty, ensuring all research is scientifically accurate, ethically aligned, and regulatory compliant.

Appendix: Animal-Free Maturity Matrix

Neurodegenerative Disease Research Roadmap

This matrix maps the progression of neurodegenerative research platforms from legacy *in vivo* reliance to human-relevant, animal-free methodologies, focusing on the specific needs of neurodegenerative modelling.

Technology Platform	Phase 1: Exploration	Phase 2: Integration	Phase 3: Validation	Phase 4: Adoption
Brain Organoids	Circuit connectivity mapping.	Standardised SOP development.	Multi-lab validation; trait modeling.	Regulatory-standardised use.
Neuro-on-a-Chip	Microfluidic architecture design.	Signalling pathway testing.	Integration into WoE frameworks.	High-throughput drug screening.
In Silico Twins	Computational network design.	AI-driven neuro-modelling.	Predictive longitudinal modeling.	Primary tool for target discovery.
Kinetic Disease Models	Molecular interaction modeling.	Protein aggregate simulations.	Data benchmarking vs. legacy models.	Core standard for efficacy testing.

Neurodegenerative Disease-Specific Applications

The following table categorises models based on their ability to replace traditional transgenic rodent pipelines for specific diseases.

Disease	Primary Animal-Free Model	Key Translational Benefit
Alzheimer's (AD)	3D iPSC-derived neurons & microglia	Recapitulates amyloid-beta/tau pathways in human genetic background.
Parkinson's (PD)	Midbrain-on-a-chip	Models dopaminergic neuron loss and Lewy body formation.
Huntington's (HD)	Human PSC-derived brain organoids	Tracks mutant Huntingtin (mHTT) propagation without rodent artifacts.
ALS	NMJ-on-chip (Neuromuscular Junction)	Models muscle atrophy and motor neuron degeneration.

Strategic Implementation Checklist

ASPA 1986 Compliance: Ensure all research strategies utilise the "Replacement" principle by verifying the lack of "scientifically satisfactory" non-animal alternatives before seeking licenses.

Context of Use (COU): Clearly define whether the model is for high-throughput screening (Level 1/2) or mechanistic physiological validation (Level 3/4).

Validation: Adopt "fit-for-purpose" standards where models are validated against human clinical data rather than rodent phenotypes, which often fail to mirror human pathophysiology.

Workforce Readiness: Institutional transition requires investment in bioinformatics and bioengineering expertise to interpret high-dimensional data from organoid and *in silico* systems.

Note: The "maturity" of a model is not defined solely by its complexity, but by its **translational validity**—how accurately it predicts human clinical outcomes. The goal of this matrix is to move beyond the false dichotomy of "animal vs. non-animal" and toward a robust, human-relevant framework for therapeutic discovery.