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NEURO-INTEGRATE

Artificial Intelligence–Assisted Design of Human Induced Pluripotent Stem Cell–Derived Neural Grafts for Guided Axonal Growth and Functional Integration

Toward Neural Circuit Reconstruction in the Brain and Spinal Cord

1. Background and Rationale

Brain and spinal cord injuries can disrupt neural circuits, impair movement and sensation, and compromise independence in daily activities. The limited regenerative capacity of the central nervous system makes restoration of lost function a major challenge in modern medicine.

Human induced pluripotent stem cells (iPSCs) provide a renewable source of neurons for cell replacement research. However, successful repair requires grafted neurons to survive, extend axons toward appropriate targets, establish suitable synaptic connections, and participate in signal transmission. Preclinical studies have demonstrated functional integration of human iPSC-derived neural progeny in experimental stroke models.

NEURO-INTEGRATE combines iPSC technology, biomaterials, and artificial intelligence (AI) to support the design and evaluation of neural grafts for functional integration. The project aims to reconstruct signal-transmitting connections and establish an experimental basis for tailoring graft design to defined circuit requirements. In the longer term, this approach could inform more precise regenerative strategies for brain and spinal cord injuries.

2. Proposed Approach and Research Hypothesis

The platform integrates three technologies:

- **iPSC-derived neurons:** Generate neuronal populations appropriate for the neural circuits under investigation.
- **Biomaterials:** Use hydrogels and microparticles to organize cells and deliver spatially controlled cues for axonal growth.
- **Artificial intelligence:** Optimize cue location, loading, and release kinetics using experimental data.

Hypothesis: Appropriate neuronal specification and controlled presentation of guidance cues can improve graft-dependent signal transmission, with functional connections persisting after exogenous cue supplementation has ended.

3. Research Objectives

- **Establish guidance responses:** Determine whether selected neurons alter axonal growth direction in response to candidate cues and investigate the underlying mechanism.
- **Identify effective designs:** Select graft configurations that improve functional transmission while maintaining neuronal viability and appropriate network activity.
- **Evaluate generalizability:** Test the workflow in spinal and cortical circuit models and determine whether spinal-model data improve AI-guided design in the cortical model.

4. Methods and Experimental Design

Step 1. Establish Two Human Neural Circuit Models

Compartmentalized neuronal cultures will model disrupted connectivity using the following populations and regions:

Symbol	Definition
A	Source neurons selectively stimulated to provide input.
G	Graft-derived neurons evaluated as a functional relay.
T	The predefined target neuronal population.
O	A predefined off-target region used to quantify axonal growth outside the intended target.

Two configurations will be examined:

- **Spinal circuit:** V2a-enriched spinal interneurons as G and motor neurons as T.
- **Cortical circuit:** Cortical projection neurons as G and a defined cortical partner population as T.

Cell identity, population composition, maturation, viability, and electrophysiological competence will be assessed. Source and target populations will be specified in advance, and support cells and culture conditions will be held consistent within each comparison.

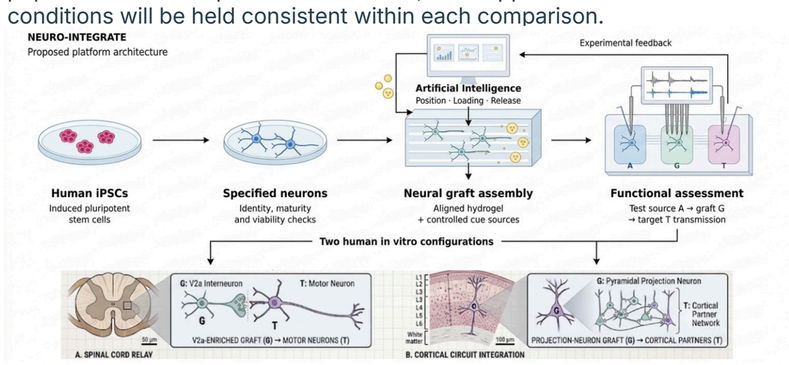


Figure 1. Proposed platform and human neural circuit models.

Step 2. Test the Mechanism of Axon Guidance

Brain-derived neurotrophic factor (BDNF) and its receptor, tropomyosin receptor kinase B (TrkB; encoded by *NTRK2*), will be investigated first in the spinal configuration. Prior work supports BDNF-directed growth of human neural stem cell-derived axons after spinal cord injury; responsiveness of the proposed neuronal populations requires separate validation.

Four conditions will be compared: *a BDNF gradient increasing toward T, a reversed gradient, spatially uniform BDNF, and a matched control without added BDNF*. Gradient reversal will help distinguish directional guidance from a general increase in growth or survival.

Measurements will include growth-cone turning angle, axonal extension rate, axon length, and neuronal survival. Full-length TrkB localization and receptor activation will be assessed. G-specific *NTRK2* knockdown followed by TrkB rescue will test receptor involvement while monitoring cellular health. Guidance responses will be validated independently in the cortical configuration.

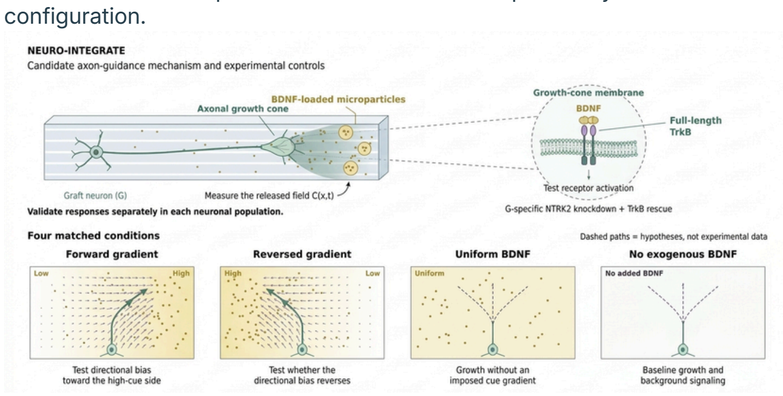


Figure 2. Candidate guidance mechanism and matched controls.

Step 3. Develop and Characterize Cue-Delivery Biomaterials

Microfluidic devices will first be used to screen spatial cue profiles. Suitable profiles will then inform hydrogels containing BDNF-releasing microparticles, building on established hydrogel–microparticle delivery approaches.

Characterization will quantify protein concentration across space and time, release kinetics, retained bioactivity, cell compatibility, material stability and degradation, and reproducibility across fabrication batches. Measured profiles will calibrate a transport model and constrain optimization to experimentally achievable designs. Release schedules will be finite and predefined.

Step 4. Optimize Designs Using AI

Bayesian optimization will select the location of cue delivery, BDNF loading, and experimentally calibrated release profiles. Predictions and uncertainty estimates will be updated after each experimental round. Bayesian active learning in biological screening provides a methodological precedent for sequential experimental design.

The primary optimization objective will be graft-dependent transmission after exogenous supplementation has ended. Neuronal survival, target-directed axonal growth, and acceptable network activity will serve as separate eligibility criteria. Neuronal type and scaffold formulation will initially remain fixed within each configuration.

AI-guided designs will be compared with a knowledge-based fixed design and random search under the same experimental budget. Selected candidates will undergo independent validation using iPSC donors and differentiation batches reserved from optimization.

- **Target preference:** A branched assay will compare G-derived axonal growth into T and O. Initially, equivalent receiving populations and swapped cue locations will control for geometry; distinct target identities will be tested subsequently. The fraction of traced axonal length in T relative to T plus O will be reported alongside absolute axonal length reaching T per surviving G neuron and axonal exit from the scaffold. Target preference will be interpreted together with total growth and survival.
- **Transfer evaluation:** In the cortical model, AI trained from scratch, AI incorporating spinal-model information, and random search will receive identical initial cortical data and equal target-model experimental budgets. Performance will be compared using independently validated candidates. Workflow generalizability and the added value of AI transfer will be evaluated separately.

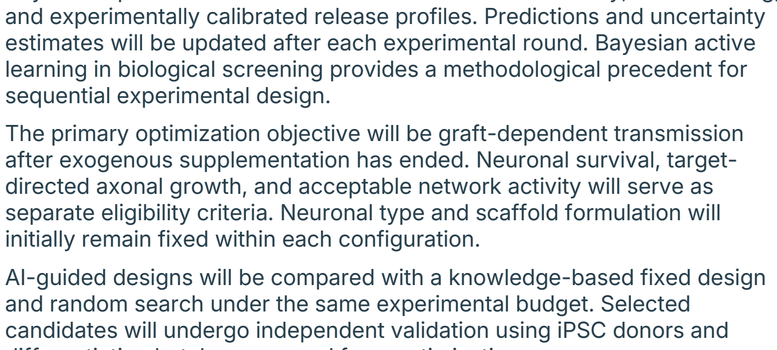


Figure 3. Experimental feedback for Bayesian optimization.

Step 5. Validate Functional Connectivity

(1) Source neurons A → Graft-derived neurons G → Target neurons T

A will be selectively stimulated while responses are recorded in T. Electrophysiological measurements will assess synaptic transmission at A–G and G–T connections. Each circuit will be evaluated through three conditions:

(2) G active → G selectively and reversibly silenced → G activity restored

The primary endpoint, R, is the difference in baseline-corrected, A-evoked target responses between the active and silenced G conditions:

$$R = \Delta T_{\text{G active}} - \Delta T_{\text{G silenced}}$$

Here, ΔT denotes the response in T following A stimulation, after subtraction of the matched unstimulated baseline, measured within a predefined recording window. R is a study-specific experimental measure of graft-dependent transmission and will be interpreted alongside synaptic evidence and pathway controls.

Controls will assess direct A–T transmission, stimulus spread, nonspecific effects of G silencing, and the intrinsic responsiveness of A and T. Comparisons will be made at matched culture ages within each configuration, after exogenous supplementation has ended, measured cue levels have returned to a predefined near-baseline range, and a prespecified follow-up interval has elapsed.

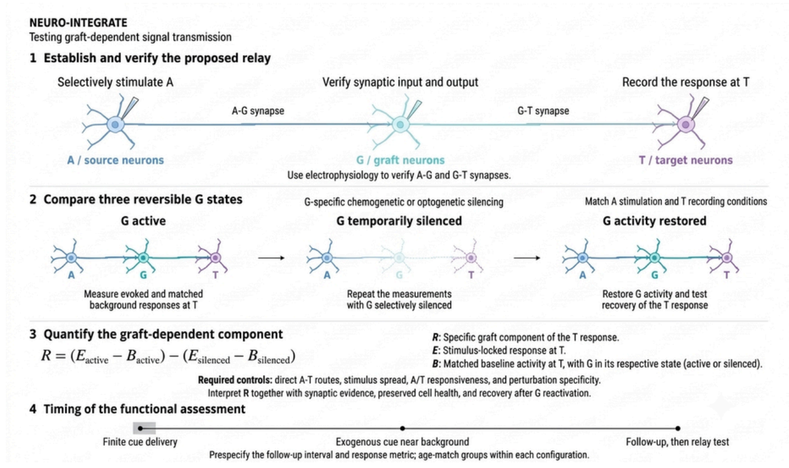


Figure 4. Functional relay validation and primary endpoint R.

5. Expected Outcomes and Evaluation Criteria

The study is expected to generate a dataset linking neuronal properties, cue-delivery profiles, and network function. Candidate designs will be selected for prototype fabrication and independent experimental validation. **Successful and unsuccessful designs** will both be retained in the dataset.

Evaluation domain	Evidence to be established
Axon guidance and TrkB signaling	Directional responses to cue profiles, receptor activation, and sensitivity to mechanistic perturbation.
Target preference	Preferential growth toward T, supported by absolute target-directed growth and neuronal survival.
Graft-dependent transmission	R, electrophysiological evidence of A–G and G–T transmission, and reversible changes during G silencing and recovery.
Network activity	Prespecified ranges of spontaneous activity and evoked responsiveness, with monitoring for aberrant network activity.
Functional persistence	Maintained transmission at the defined post-supplementation assessment.
AI performance and transfer	Independently validated performance against budget-matched comparators and evidence for or against a benefit from spinal-to-cortical transfer.

Axonal abundance and synaptic markers will be interpreted alongside functional transmission measurements to establish their physiological relevance.

Statistical rigor

Statistical rigor: Pilot data will estimate variability and inform the confirmatory sample size. Circuits will be randomly assigned to conditions, analyses blinded, and effect estimates reported with 95% confidence intervals to express uncertainty. The independently treated circuit is the experimental unit; images, axons, and repeated stimulation trials are nested measurements. Analyses will account for donor and differentiation-batch effects.

6. Proposed Contribution and Application Potential

The proposed contribution is a workflow that connects manufacturable guidance designs with experimental evidence of functional integration. It combines axon guidance, controlled cue delivery, and AI-guided selection, with explicit tests of post-supplementation persistence and transfer between circuit configurations.

- **Anticipated outputs:** Human neural circuit models, a dataset supporting graft-design optimization, a functional validation workflow, and a prototype neural graft incorporating guidance cues.
- **Application potential:** The platform could provide a research foundation for reconstructing motor pathways after spinal cord injury and supporting the integration of replacement neurons into cortical networks after stroke. Initial studies will use human cell-based models, which do not fully reproduce inflammation, scarring, vascular disruption, or immune responses. Therapeutic development will require further preclinical evaluation of efficacy, residual pluripotent cells, uncontrolled proliferation, aberrant connectivity, and long-term safety.

7. Conclusion

NEURO-INTEGRATE proposes an AI-assisted strategy for guiding human iPSC-derived neurons toward functional integration in models of disrupted neural circuits, combining controlled cue delivery with experimental feedback to optimize graft design. Its value will be assessed through graft-dependent signal transmission, persistence after exogenous cue supplementation has ended, and reproducibility across spinal and cortical configurations. These findings could provide an experimental foundation for developing neural repair strategies for spinal cord injury and stroke.

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