RESEARCH PLAN

OVERVIEW

Dopamine research has progressed at two extremes. At the molecular level, we understand how D1 and D2 receptors couple to intracellular signaling cascades, how dopamine is synthesized and cleared, and how individual neurons respond to dopaminergic input. At the systems level, we observe the consequences of dopamine disruption: the motor deficits of Parkinson's disease, the cognitive impairments of prefrontal hypodopaminergia, and the reward dysregulation of addiction. What remains largely unexplored is the middle ground, the circuit-level dynamics through which populations of neurons collectively transform dopaminergic signals into organized network activity. Human midbrain organoids, recorded on high-density multi-electrode arrays, offer new opportunities to investigate these dynamics in human tissue.

SIGNIFICANCE

This proposal tests specific predictions from four major theoretical frameworks (Grace's tonic/phasic dynamics, Eshel's GABA-DA local computation, Seamans' D1/D2 stability-flexibility model, and Frank's Go/NoGo reinforcement learning model) that have never been examined at the mesoscale in human tissue. Each framework generates clear predictions about how dopamine should modulate collective neural activity: tonic dopamine should alter network responsivity (Grace, 1991), local GABA-DA interactions should shape midbrain output (Eshel et al., 2015), D1 activation should stabilize network states while D2 activation promotes flexibility (Seamans & Yang, 2004), and progressive dopamine loss should asymmetrically disrupt D1- and D2-mediated network processes (Frank, 2005). By testing these predictions pharmacologically and computationally in human midbrain organoids, this work bridges the gap between molecular mechanisms and systems-level function.

INNOVATION

We introduce a combined pharmacological and computational approach for characterizing dopaminergic modulation at the network level in human tissue. Multi-electrode array recordings capture population-level dynamics that single-cell methods cannot access. Our computational pipeline (adaptive burst detection, temporal correlation analysis, principal component analysis, and hierarchical clustering) overcomes a critical analytical challenge: standard pairwise correlation methods become unreliable when neurodegeneration reduces active neuron counts, precisely the condition we study. By analyzing coordination patterns at the burst level rather than tracking individual neurons, we can characterize network organization even as the circuit degrades. Linear mixed-effects models account for the substantial organoid-to-organoid variability inherent to these preparations.

Assembloid co-culture with cortical and striatal organoids enables progressive scaling of circuit complexity (from isolated midbrain tissue to multiregional circuits) testing whether dopaminergic network dynamics change in predictable, framework-consistent ways when projection targets are provided. This systematic approach has not been attempted in human tissue.

APPROACH

All experiments employ human midbrain organoids containing dopaminergic (TH+, DAT+), GABAergic, and glutamatergic neurons, recorded on the MaxOne/MaxTwo high-density MEA platform. Pharmacological perturbations use dopamine-HCl (endogenous agonist modeling volume transmission), haloperidol (D2/D3 antagonist), SCH23390 (D1/D5 antagonist), quinpirole (D2/D3 agonist), 6-OHDA (selective dopaminergic neurotoxin), and rotenone (mitochondrial complex I inhibitor). Off-target effects for each agent are characterized and addressed through dose-response calibration, RT-qPCR quantification of off-target receptor expression, replication with alternative agents, and combined blockade controls. Dopamine production is confirmed by ELISA, and cellular composition is validated by immunohistochemistry and RNA sequencing. Preliminary 6-OHDA data demonstrate that the system produces detectable, quantifiable

treatment effects and that the computational pipeline can capture network reorganization following dopaminergic lesion.

BACKGROUND

(1) DOPAMINE AS A NEUROMODULATOR

Dopamine functions as a neuromodulator rather than a classical neurotransmitter (Seamans & Yang, 2004; Beaulieu & Gainetdinov, 2011). Unlike glutamate or GABA, dopamine does not directly drive neuronal firing through rapid ionotropic receptor activation. Instead, it modulates how networks respond to other inputs, altering the gain, timing, and plasticity of glutamatergic and GABAergic transmission. Dopaminergic effects are therefore state-dependent: they require ongoing activity to modulate. This distinction is fundamental to interpreting our experiments, because bath-applied dopamine should not generate activity de novo but should change the character of existing network dynamics.

Dopamine signaling operates primarily through volume transmission rather than point-to-point synaptic transmission (Rice et al., 2011). Released from en passant varicosities along extensively branching axons, dopamine diffuses through the extracellular space to reach receptors distributed across a tissue volume. A single nigrostriatal neuron forms approximately 10^5 release sites, and most dopamine receptors and transporters are located extrasynaptically (Yung et al., 1995; Smiley et al., 1994). Bath-applied dopamine therefore recapitulates the physiological mode of dopaminergic signaling (diffusion across a tissue volume) more accurately than discrete synaptic stimulation.

(2) RECEPTOR PHARMACOLOGY AND D1/D2 OPPONENCY

Dopamine receptors fall into two families with opposing downstream effects (Beaulieu & Gainetdinov, 2011). D1-like receptors (D1 and D5) couple to Gαs/olf proteins, stimulating adenylyl cyclase to increase intracellular cAMP and activate protein kinase A. D2-like receptors (D2, D3, D4) couple to Gαi/o proteins, inhibiting adenylyl cyclase while also activating GIRK potassium channels and inhibiting voltage-gated calcium channels. This molecular machinery is identical regardless of the brain region receiving dopaminergic innervation, and the same receptor subtypes and signaling cascades operate in striatum, cortex, and midbrain.

D1 and D2 receptor activation generally produce opposing effects on target neurons (Beaulieu & Gainetdinov, 2011; Cools & D'Esposito, 2011). D1 activation typically increases neuronal excitability, promotes the stability of active representations, and facilitates long-term potentiation. D2 activation typically decreases excitability, promotes flexibility and switching between states, and facilitates long-term depression. The balance between D1 and D2 signaling determines the network's operating mode. A universal feature of this balance is the inverted-U relationship between dopamine levels and functional performance: too little dopamine impairs function, optimal levels support it, and excessive dopamine impairs it again (Cools & D'Esposito, 2011). These conserved principles (identical receptor pharmacology, D1/D2 opponency, and the inverted-U dose-response) justify testing dopaminergic modulation in any preparation that contains functional dopaminergic neurons and expresses these receptors.

(3) TONIC AND PHASIC RELEASE

Dopamine release occurs through two independent mechanisms (Grace, 1991). Tonic release results from low-frequency pacemaker firing (~4 Hz), producing sustained extrasynaptic dopamine accumulation that sets baseline receptor occupancy. Because D2 receptors have higher affinity for dopamine than D1 receptors, tonic levels preferentially occupy D2 receptors (Dreyer et al., 2010). Phasic release results from burst firing (15–30 Hz) triggered by behaviorally relevant stimuli, producing transient high-concentration spikes that additionally activate lower-affinity D1 receptors. Floresco et al. (2003) validated this framework experimentally by showing that pallidal inhibition regulates population activity (tonic) while

pedunculopontine input regulates burst firing (phasic). These are two dissociable afferent systems controlling the two modes of release.

D2 autoreceptors on dopaminergic neurons provide negative feedback that regulates the dopamine system (Ford, 2014). Somatodendritic autoreceptors in the VTA and SNc respond to locally released dopamine by activating GIRK2 channels, hyperpolarizing the neuron and reducing its firing rate. Hikima et al. (2021) demonstrated that this is truly "auto"-inhibition: each neuron is inhibited by its own released dopamine, not merely by spillover from neighbors. Cragg & Greenfield (1997) revealed regional heterogeneity, with strong D2 autoinhibition in the SNc and weaker control in the VTA. These autoreceptor dynamics are directly relevant to our system, where bath-applied dopamine should engage D2 autoreceptors on organoid dopaminergic neurons.

(4) THEORETICAL FRAMEWORKS

Grace's tonic/phasic framework. Grace (1991) established that tonic dopamine sets baseline receptor occupancy and—critically—modulates the system's responsivity to subsequent phasic signals. This framework predicts that bath-applied dopamine (modeling tonic elevation) should alter how our organoid circuits respond to subsequent inputs, and that D2 blockade should disinhibit dopaminergic neurons by removing autoreceptor feedback.

Eshel's GABA-DA local computation. Eshel et al. (2015) showed that VTA GABA neurons perform local computations on DA neuron output, providing an "expected reward" signal that DA neurons subtract to compute prediction errors. Cohen et al. (2012) confirmed that VTA DA and GABA neurons encode fundamentally different signals: DA neurons carry phasic prediction error while GABA neurons carry sustained expectation signals. This framework applies directly to our system, which contains both DA and GABA neurons and records their integrated interaction.

Seamans' D1/D2 stability-flexibility model. Seamans & Yang (2004) synthesized conflicting findings about dopamine's prefrontal effects into a unified framework: D1 activation enhances signal-to-noise ratio by boosting strong inputs and suppressing weak ones (stability), while D2 activation produces effects that generally promote flexibility and transitions between network states. Durstewitz & Seamans (2008) extended this computationally, proposing that PFC networks operate in two attractor states governed by D1/D2 balance. Fujimoto et al. (2025) validated these principles at the whole-brain scale: D1 blockade diminished functional connectivity broadly, while D2 blockade enhanced it. Although this framework was developed in cortical target tissue, the receptor pharmacology is identical in midbrain, allowing us to test whether analogous network signatures emerge in the source tissue.

Frank's Go/NoGo reinforcement learning model. Frank (2005) formalized how D1 and D2 receptors in striatum implement opponent learning signals: phasic DA bursts activate D1 receptors to strengthen Go pathway responses, while DA dips activate D2 receptors to strengthen NoGo pathway responses. Frank et al. (2004) validated this behaviorally in Parkinson's patients, showing that medication status asymmetrically impaired Go versus NoGo learning. While developed for striatal targets, the underlying D1/D2 opponency is conserved across brain regions, and our system can test whether this fundamental opposition manifests in midbrain source tissue.

(5) NEUROTOXIN MODELS OF DOPAMINERGIC DYSFUNCTION

Two complementary neurotoxin models provide distinct windows into dopaminergic circuit dysfunction. 6-Hydroxydopamine (6-OHDA), a catecholaminergic neurotoxin selectively taken up by DAT and NET, concentrates within catecholaminergic neurons and generates reactive oxygen species that destroy them (Bové & Perier, 2012). In organoid preparations, 6-OHDA treatment (100–200 µM for 24–48 hours) selectively destroys TH-positive neurons while preserving overall tissue architecture. This models late-stage Parkinson's disease, after substantial dopaminergic neuron loss has already occurred.

Rotenone, a mitochondrial complex I inhibitor, crosses cell membranes freely but preferentially damages dopaminergic neurons because of their high metabolic demands and lower mitochondrial reserve (Greenamyre et al., 2003; Betarbet et al., 2000). Chronic low-dose exposure (1–50 nM) with repeat dosing every 48 hours over 7 days models prodromal Parkinson's disease, the period of progressive dysfunction before catastrophic neuron loss. The expected trajectory is biphasic: acute functional dysfunction followed by commitment to cell death. Together, these models address a central question from Frank's framework: how does progressive loss of dopaminergic modulation alter the D1/D2 balance that governs network dynamics?

(6) THE EXPERIMENTAL SYSTEM

Human midbrain organoids contain dopaminergic neurons (TH+, DAT+, VMAT2+), GABAergic neurons, glutamatergic neurons, and astroglia (Sharf et al., 2022). Spontaneous network activity detected on multi-electrode arrays exhibits characteristic bursting patterns and network synchronization events. Our organoids lack several features present in vivo: pallidal GABAergic inhibition that controls which DA neurons fire tonically (approximately 50% are held hyperpolarized in vivo; Floresco et al., 2003), pedunculopontine glutamatergic input that drives burst firing, and the projection targets whose retrograde signals may shape DA neuron identity (Lammel et al., 2008). These differences mean that baseline activity patterns may differ from in vivo, potentially all capable DA neurons fire spontaneously, and burst firing may arise from local excitatory input rather than brainstem afferents. However, this isolation also allows us to study intrinsic midbrain network dynamics that extrinsic inhibition normally veils.

DA neuron heterogeneity in our system reflects the in vivo condition. Midbrain DA neurons exhibit diverse electrophysiological properties that vary by projection target (Lammel et al., 2008), and traditional identification criteria (Ih current, action potential duration, firing rate, D2 agonist inhibition) are insufficient to reliably distinguish DA from non-DA neurons (Margolis et al., 2006). Our MEA bursting features therefore represent the emergent activity of a mixed network, which parallels how in vivo midbrain circuits respond to dopaminergic perturbation as an integrated system. The relevant question is not which individual cells are dopaminergic, but how the heterogeneous circuit responds collectively to dopaminergic modulation.

AIM 1: CHARACTERIZE INTRINSIC DOPAMINERGIC MODULATION IN MIDBRAIN ORGANOID CIRCUITS

The first aim determines how dopamine modulates the intrinsic network dynamics of midbrain tissue—where dopaminergic neurons reside alongside the GABAergic and glutamatergic populations they interact with locally. Even without projection targets, midbrain organoids contain the cellular components of the local GABA-DA interaction that Eshel et al. (2015) demonstrated performs computations on DA neuron output in the VTA. Grace's framework (1991) predicts that tonic dopamine elevation should alter network responsivity by shifting receptor occupancy and engaging autoreceptor feedback. These predictions can be tested without assumptions about projection target identity or downstream connectivity. However, because our system differs fundamentally from in vivo midbrain (lacking pallidal inhibition, pedunculopontine input, and target-derived signals) this aim begins with systematic characterization and calibration.

(1.1) Molecular and Biochemical Characterization

To establish which dopaminergic receptors are present and at what abundance, I will perform comprehensive receptor characterization from at least three independent differentiation batches (minimum n = 9 organoids total). Immunohistochemistry will verify the presence and spatial distribution of TH-positive, GAD67-positive, and vGlut1/2-positive neurons, establishing the cellular composition of the network being recorded. RT-qPCR will quantify mRNA expression of D1 and D2 receptor subtypes, dopamine transporter, and VMAT2. ELISA will measure baseline dopamine concentration in conditioned media. Fluorescent ligand binding will provide quantitative estimates of D1 and D2 receptor density and spatial distribution. Success criteria include detectable TH+ neurons comprising at least 10% of the

neuronal population with co-localization of at least one additional dopaminergic marker, detectable D1 and D2 receptor mRNA, measurable dopamine in conditioned media, and quantifiable fluorescent ligand binding. Timeline: Months 1–3.

(1.2) Baseline Electrophysiology and System Calibration

I will record spontaneous activity from midbrain organoids on the MaxTwo MEA platform (minimum n = 12 organoids from independent batches) and apply adaptive RMS-based burst detection to characterize population burst properties: frequency, duration, peak firing rate, interburst interval, and burst-to-burst variability. Dose-response curves for each pharmacological agent will be established before any hypothesis-driven experiment. For dopamine-HCl, concentrations from 100 nM to 100 µM (half-log steps) will identify the range producing detectable network modulation without saturating the response. For haloperidol and SCH23390, concentrations from 300 nM to 30 µM will define the dose-response relationship. Off-target considerations are addressed explicitly: haloperidol at concentrations producing complete D2 occupancy also engages sigma-1 receptors at comparable affinity (Kroeze et al., 2003), and SCH23390 is a potent 5-HT2C agonist (Millan et al., 2001). I will quantify 5-HT2C expression by RT-qPCR to assess the serotonergic confound risk. Timeline: Months 1–4.

(1.3) Testing Predictions from Grace's Tonic/Phasic Framework

Prediction 1 (Autoreceptor-mediated modulation). DA perfusion at the tonic-range concentration identified in 1.2 should engage D2 autoreceptors on DA neurons, producing autoinhibition (Hikima et al., 2021; Ford, 2014). At the network level, this predicts a shift in burst dynamics—not necessarily suppression of all activity, since dopamine is a neuromodulator rather than a neurotransmitter, but a change in the temporal patterning and coordination of population bursts. If this autoinhibition is D2-mediated, haloperidol pretreatment should block or attenuate the effect. Experimental design: baseline recording (30 min), DA perfusion (60 min), washout (30 min); repeated with haloperidol pretreatment in a separate series. Within-organoid paired comparisons using the linear mixed-effects framework.

Prediction 2 (GABA-DA interaction). Because our organoids contain both DA and GABA neurons, DA perfusion should affect both populations (DA neurons via D2 autoreceptors, GABA neurons via postsynaptic D1 and D2 receptors) and the network output should reflect their integrated interaction. To dissect these contributions, I will compare the effect of DA perfusion alone, DA perfusion after D2 blockade with haloperidol (autoreceptor feedback removed, D1 effects preserved), and DA perfusion after D1 blockade with SCH23390 (D1 effects removed, autoreceptor effects preserved). If functional GABA-DA interactions are present, these three conditions should produce qualitatively distinct network signatures, not simply graded versions of the same response.

Prediction 3 (Responsivity modulation). Grace (1991) proposed that tonic DA levels set the gain of the system. To test this, I will deliver electrical stimulation through the MEA to provide a standardized input before and after DA perfusion. Stimulus parameters (biphasic current pulses, single pulses and trains at 4 Hz and 20 Hz to mimic tonic and phasic patterns) will be calibrated to produce reliable evoked network responses at baseline. If tonic DA elevation modulates system responsivity, the amplitude, latency, or coordination pattern of the evoked response should change after DA perfusion and should be partially reversible by washout.

Success criteria: DA perfusion produces a statistically significant change in at least two burst metrics; haloperidol pretreatment significantly modifies the DA effect; the three pharmacological conditions produce distinguishable network signatures; electrical stimulation produces reliable evoked responses. Timeline: Months 3–8.

(1.4) Deeper Characterization and Assembloid Development

As experimentally relevant features emerge, single-cell RNA-seq on a subset of organoids will provide a transcriptomic census: the proportions of neuronal subtypes, which DA neuron subtypes are present (SNc-like versus VTA-like), and whether D2 autoreceptor expression (DRD2 + GIRK2/KCNJ6 co-expression) confirms the biological basis for the predictions tested in 1.3. To test whether DA neuron functional

properties are shaped by projection targets (as Lammel et al. (2008) demonstrated in vivo) I will generate assembloid co-cultures by fusing midbrain organoids with cortical organoids. Comparing pharmacological responses in midbrain-only versus midbrain-cortical preparations will reveal whether projection target identity influences how DA modulation manifests at the network level. Timeline: Months 6–14.

AIM 2: INVESTIGATE D1/D2 OPPONENCY AND NETWORK STATE DYNAMICS

The second aim determines whether the D1-stability/D2-flexibility principle (established in prefrontal cortex (Seamans & Yang, 2004; Durstewitz & Seamans, 2008) and validated at the whole-brain scale (Fujimoto et al., 2025)) manifests as distinct, opposing network states in midbrain source tissue. This aim tests a fundamental question: is D1/D2 opponency an intrinsic property of any tissue expressing these receptors, or does it require the specific cytoarchitecture of target circuits to emerge? This aim builds on the dose-response curves from Aim 1 but shifts focus from dopamine's general modulatory effects to the receptor-specific mechanisms that determine how dopamine shapes network states.

(2.1) Establishing D1/D2 Network Signatures

Using the calibrated concentrations from Aim 1, I will perform selective receptor blockade experiments: baseline recording (30 min), selective antagonist application (60 min), dopamine perfusion in the presence of the antagonist (60 min), washout (30 min). Durstewitz & Seamans (2008) predict that D1-dominated states should produce stable attractor dynamics (high reproducibility, low burst-to-burst variability, strong temporal coordination), while D2-dominated states should produce flexible, variable dynamics. D2 blockade with haloperidol (isolating D1 effects) should therefore drive the network toward stability; D1 blockade with SCH23390 (isolating D2 effects) should drive it toward variability. PCA applied to burst correlation matrices will quantify whether D1-dominated and D2-dominated conditions occupy distinct regions of network state space. Combined D1 + D2 blockade provides a critical control: if D1/D2 opponency is present, removing both arms simultaneously should produce a qualitatively different state than removing either alone.

(2.2) Testing the Inverted-U at the Mesoscale

Graded dopamine concentrations across the full range established in 1.2 will be applied to organoids while measuring network stability, operationalized as the inverse of burst-to-burst variability in temporal coordination patterns. If the inverted-U holds, there should be a concentration that maximizes network stability, with reduced stability at both lower and higher concentrations. This relationship has been demonstrated in PFC single neurons (Vijayraghavan et al., 2007) and in cognitive performance (Cools & D'Esposito, 2011) but has never been tested at the network level in midbrain tissue. The optimal concentration identified here becomes a critical reference point for all subsequent experiments.

(2.3) Electrical Stimulation of Network State Transitions

To probe dynamics faster than pharmacological manipulation allows, I will use patterned electrical stimulation to drive network state transitions and measure how D1/D2 balance affects the ease, speed, and completeness of those transitions. Distinct coordination states identified from baseline clustering analysis will serve as targets. Under D2 blockade (D1-dominated), Durstewitz & Seamans (2008) predict that the network should resist transitions; under D1 blockade (D2-dominated), transitions should be easier and less stable. This experiment tests whether dopamine receptor balance governs the dynamics of network state transitions, not just their static properties.

(2.4) Assembloid Co-Culture: Introducing Mesocortical Projection Targets

The D1-stability/D2-flexibility framework was developed in prefrontal cortex, a mesocortical projection target. Midbrain-cortical assembloids generated by fusing midbrain organoids with dorsal forebrain organoids will test whether providing cortical targets alters how D1/D2 opponency manifests at the network level. If the Seamans framework predicts correctly, the cortical compartment should amplify

D1/D2 opponency. If the midbrain-only organoid already shows robust opponency, the assembloid may not add substantially, suggesting that opponency is intrinsic to the source tissue. Success criteria: D2 and D1 blockade produce statistically distinguishable, opposing effects on burst metrics; graded DA concentrations produce a non-monotonic (inverted-U) relationship with network stability; patterned stimulation dynamics are modified by receptor blockade. Timeline: Months 4–18.

AIM 3: MODEL DOPAMINERGIC CIRCUIT DYSFUNCTION AND THE NIGROSTRIATAL GO/NOGO SYSTEM

The third aim addresses the most translationally relevant question: how does dopaminergic neuron loss transform network dynamics, and can the computational framework developed for the nigrostriatal Go/NoGo system (Frank, 2005) illuminate what happens to the dopamine-producing circuit when that circuit is destroyed? Frank's framework predicts that dopamine depletion should impair D1-mediated Go processes while relatively preserving D2-mediated NoGo processes, and that pharmacological restoration should differentially rescue these. This aim incorporates preliminary 6-OHDA data and extends the investigation with chronic rotenone exposure, pharmacological rescue, and assembloid co-culture with striatal targets.

(3.1) Acute Dopaminergic Ablation: 6-OHDA Model

Midbrain organoids treated with 6-OHDA (50, 100, or 200 µM for 24–48 hours; minimum n = 12 treatment, n = 6 vehicle control) will model late-stage Parkinson's disease. Treatment selectivity will be validated with TH and MAP2 immunohistochemistry and dopamine ELISA. The central question is whether 6-OHDA produces network reorganization (changed coordination patterns and altered state dynamics) rather than simple suppression (proportional reduction in all activity). This distinction matters for therapeutic strategy: reorganized circuits require different approaches than replacing lost function. Preliminary data support reorganization, showing increased burst-to-burst variability and loss of stereotyped temporal coordination following 6-OHDA treatment. Timeline: Months 1–6.

(3.2) Chronic Dopaminergic Degeneration: Rotenone Model

Chronic low-dose rotenone exposure (1–50 nM, repeat dosing every 48 hours for 7 days) models prodromal Parkinson's disease. Because rotenone inhibits mitochondrial complex I in all cells, dopaminergic neurons are preferentially vulnerable owing to their high oxidative metabolic demands (Surmeier et al., 2017). Daily MEA recordings will capture the trajectory of network degradation. The central prediction is that this trajectory should be biphasic—an initial compensatory phase (Days 1–3) followed by decompensation (Days 4–7) as compensatory capacity is exhausted. A significant quadratic term in the linear mixed-effects model would support this hypothesis. Features shared between the 6-OHDA and rotenone models likely reflect core consequences of dopaminergic loss, while features unique to rotenone likely reflect broader mitochondrial toxicity. Timeline: Months 6–12.

(3.3) Pharmacological Rescue and D1/D2 Dissection Post-Lesion

Frank's framework predicts that dopamine replacement should rescue D1-mediated network features while potentially impairing D2-mediated features, mirroring the clinical observation that L-DOPA alleviates motor symptoms but impairs learning dependent on dopamine dips (Frank et al., 2004). After 6-OHDA treatment, bath application of dopamine with and without selective receptor blockade will test whether the rescue effect is receptor-specific: rescue with DA + D2 blockade (isolating D1-mediated rescue) versus DA + D1 blockade (isolating D2-mediated rescue). L-DOPA will also be tested; because it requires conversion to dopamine by AADC in DA neurons, L-DOPA should be less effective than direct dopamine application if 6-OHDA has destroyed the conversion machinery, a prediction with direct translational relevance. Timeline: Months 8–14.

(3.4) Assembloid Co-Culture: Introducing Nigrostriatal Projection Targets

Midbrain-striatal assembloids generated by fusing midbrain organoids with lateral ganglionic eminence-derived organoids enable the most direct test of the Go/NoGo framework in human tissue. If dopaminergic projections extend into the striatal compartment, 6-OHDA treatment should produce asymmetric effects: the midbrain shows the reorganization characterized in 3.1, while the striatal compartment shows changes consistent with disrupted D1/D2 balance on medium spiny neurons. Patterned electrical stimulation in the striatal compartment can test whether Go-like and NoGo-like response patterns are differentially affected by dopamine depletion, as Frank's model predicts. Timeline: Months 12–20.

(3.5) Comparative Analysis Across Disease Models

Direct comparison of 6-OHDA (acute ablation) and rotenone (chronic degeneration) results using linear mixed-effects models with treatment type as a between-group factor will distinguish general consequences of dopaminergic loss from mechanism-specific features. Success criteria across Aim 3: 6-OHDA produces selective ablation (>50% TH+ reduction, <20% MAP2+ reduction) with statistically significant network reorganization; rotenone produces a detectable trajectory distinguishable from vehicle controls; DA rescue partially reverses at least one 6-OHDA-induced network change with receptor-specific effects; assembloids show functional integration with distinguishable effects in midbrain versus striatal compartments. Timeline: Months 14–18.

TIMELINE AND FEASIBILITY

The three aims build on each other while maintaining independent value. Aim 1 provides the foundational characterization, dose-response calibration, and baseline metrics that Aims 2 and 3 require. However, the 6-OHDA experiments in Aim 3 proceed in parallel using existing preliminary data, and the pharmacological experiments in Aim 2 begin as soon as dose-response curves from Aim 1 are established. Months 1–6: Aim 1 molecular characterization and baseline electrophysiology, concurrent with completing 6-OHDA characterization. Months 3–12: Aim 1 pharmacological hypothesis testing and Aim 2 D1/D2 opponency experiments, concurrent with rotenone optimization. Months 6–14: deeper characterization, stimulation experiments, and pharmacological rescue. Months 10–20: assembloid experiments across aims and comparative analysis. Months 18–24: integration, manuscript preparation, and follow-up experiments.

Feasibility is supported by established experimental infrastructure. MEA recording, organoid culture, pharmacological perturbation protocols, and computational analysis pipelines are all operational. Preliminary 6-OHDA data validate that the system produces detectable treatment effects. The Sharf Lab has expertise in organoid electrophysiology (Sharf et al., 2022) and access to the MaxOne/MaxTwo MEA platforms. The Braingeneers consortium provides computational resources and collaborative expertise in neural data analysis.

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