

Effects of Dopaminergic Toxicity on Spatiotemporal Network Dynamics in Parkinson's-Induced Human Midbrain Organoids

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Introduction

Significance for Brain Organoid Research

- **Brain organoids** - 3D brain-like structures grown from iPSC-based human stem cells in the lab
- Organoids self-organize into neural tissues imitating the brain
- Able to be analyzed through imaging, gene expressions, and most importantly, electrophysiology recordings (through Microelectrode Arrays)



Figure 1. CMOS-based microelectrode array chip. These devices contain thousands of miniaturized amplifiers used to triangulate the electrical activity of single neurons within millimeter-sized organoid tissue (Photo taken by Sharf Lab, UCSC)

- High-density **CMOS MEAs** enable:
 - Precise localization of neuronal spikes
 - Multiple concurrent recordings of different 'wells'
 - Investigation of network-level phenomena that are not accessible in other **in vivo models**

Parkinson's Disease Modeling Approach

- **Parkinson's disease (PD):** Progressive neurodegenerative disorder driven by loss of **dopaminergic neurons** in the substantia nigra
- **Clinical impact:** Causes motor symptoms (bradykinesia, rigidity, tremor) and non-motor symptoms (mood and sleep issues); affects **>10 million** people worldwide
- **Pathology:** Associated with α -synuclein aggregates (Lewy bodies) and cellular dysfunction (e.g., mitochondrial disruption) that contributes to neuron death
- **Problem – Current Limitation of PD models:**
 - Post-mortem human tissue: scarce; reflects late-stage/irreversible changes
 - Animal models: useful but limited by species differences
 - 2D cell cultures: keep patient genetics but often lack realistic multicellular **network organization**
- **Why Organoids?:** Human **midbrain organoids** provide a human-relevant 3D dopaminergic network model for early PD-like dysfunction.
- **Why 6-OHDA?:** The drug **6-OHDA** induces PD-like dopaminergic toxicity and is associated with oxidative stress and mitochondrial dysfunction, attributing to its common use in many in vitro tests of Parkinson's Disease

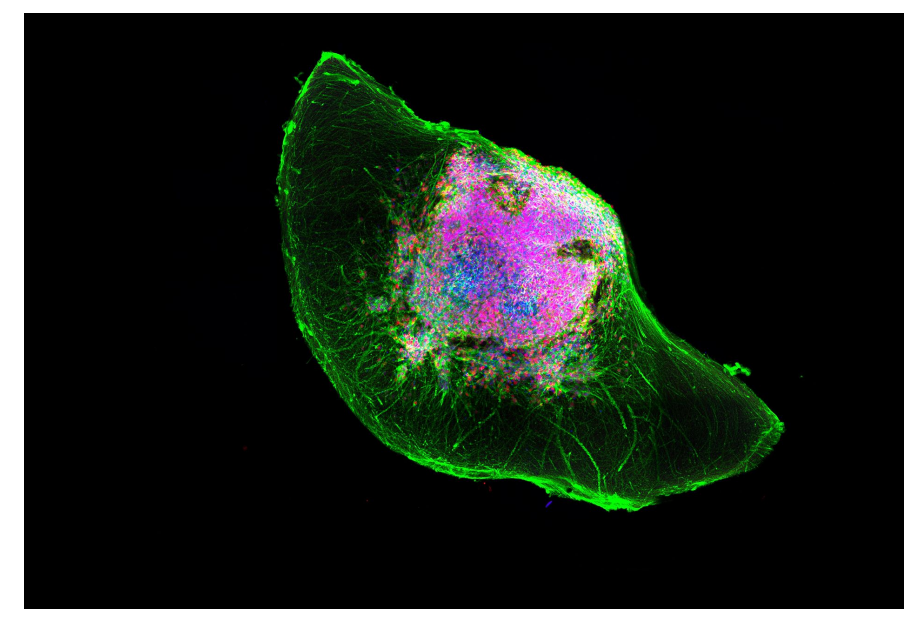


Figure 2: An immunofluorescence image of a midbrain organoid. Dopaminergic neurons important in Parkinson's disease shown in green. Image courtesy of OrganoTherapeutics

Research Question: Can early electrophysiological signatures of PD-like dysfunction be detected before overt degeneration in human midbrain organoids?

Project Goal: Determine whether PD-like perturbations induce detectable **network-level electrophysiological dysfunction** prior to obvious neuronal loss.

Hypothesis

Dopaminergic toxicity (6-OHDA) will shift brain organoid network dynamics toward PD-consistent dysfunction, reflected in:

1. decreased network complexity (reduced synergy / increased redundancy)
2. weakened topographical organization (changes in hub centrality, etc.)
3. impaired directed information flow efficiency

Then, due to clear differences, we predict an unsupervised ML model can learn from healthy network dynamics and detect PD-like deviations.

Methods

Data processing (QC + Standardization)

- Screen 6 wells/sample; exclude wells with **low spike counts, irregular temporal activity**
- Sample size: Control Organoids, n=18, PD induced Organoids, n=18
- Optimize neuronal firing locations through **KiloSort 2** spike sorting
- Spatial normalization:

$$d^* = \frac{d}{d_{NN, med}}$$

- **Outputs:** spike trains, (normalized) coordinates, metadata

Construct Functional Connectivity Graph

- Compute pairwise firing differences
- Construct **STTC Matrix** with a 5ms coincidence window
- Standardize through z-scoring across recording duration

$$Z_{ij} = \frac{W_{ij} - \mu_W}{\sigma_W}$$

- Shift spikes by 5 - 10ms (rate-bias control) to reduce false-positive correlations between bursts

Network Complexity (Higher-Order Interactions)

- For each neuron, define local neighborhoods using **k-Nearest Neighbors** (k = 2, 3, 4, 5)
- Estimate entropies (Gaussian-copula KDE) and compute **O-information** for each k
 - $\Omega > 0$: redundancy
 - $\Omega < 0$: synergy

$$\Omega(X_1, \dots, X_n) = \sum_{i=1}^n H(X_i) - (n-1)H(X_1, \dots, X_n)$$

H(X): entropy

- Identify which spatial scales show strongest redundancy/synergy differences
- **Endpoints:** O-information coefficients (PD vs Control across multiple days)

Topographical Structure (Spatial Organization)

- Compare **connectivity vs. distance** profiles (short vs. long-range organization) using d^* calculations
- **Hub Centrality:** total connection values to each node $\rightarrow$ identify top ~15 nodes

$$C_i = \frac{2E_i}{k_i(k_i - 1)}$$

- **Rich Club:** measure node preference of 'hub nodes' vs. random networks

$$L = \frac{1}{N(N-1)} \sum_{i \neq j} d(i, j)$$

- **Small World Structure:** compare clustering coefficients with path lengths

$$\sigma = \frac{C/C_{rand}}{L/L_{rand}}$$

Information-Flow Patterns (Directed Dynamics)

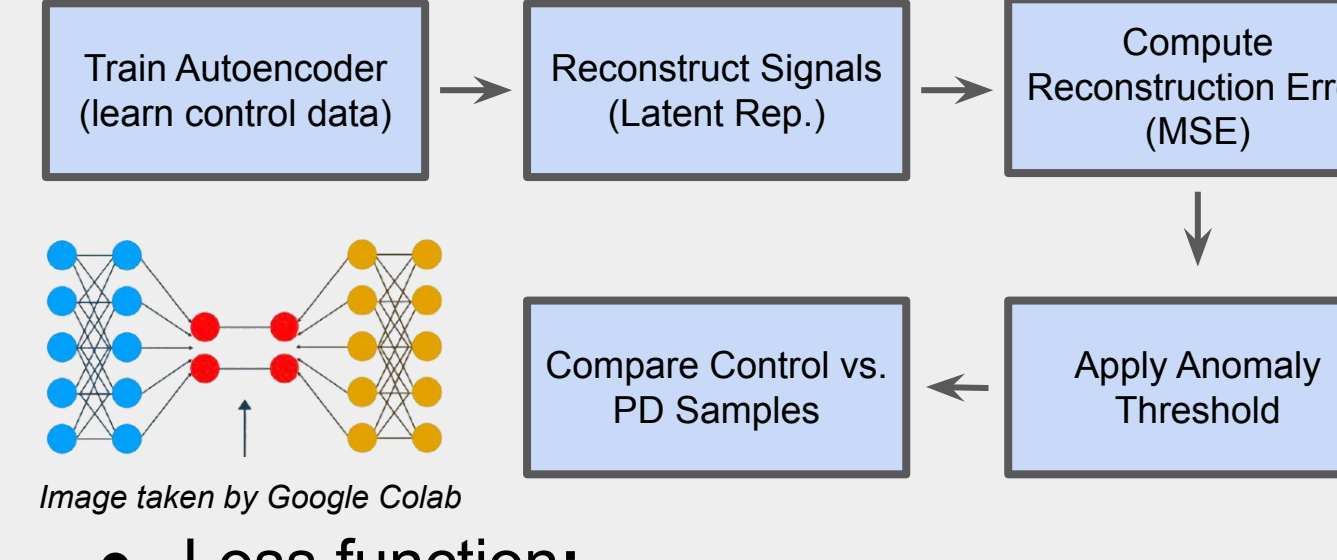
- Compute **Transfer Entropy (TE)** to measure directed predictive influence ($X \rightarrow YX \rightarrow Y$)

$$T_{X \rightarrow Y} = \sum p(y_{t+1}, y_t, x_t) \log \frac{p(y_{t+1} | y_t, x_t)}{p(y_{t+1} | y_t)}$$

- Apply spatial mask (**Delaunay/Gabriel graph**) $\rightarrow$ keep only plausible local edges
- Build **directed TE graph** (weights + distance)
- Quantify flow in two different metrics:
 - Random flow: Markov chain $\rightarrow$ **mean first-passage time**
 - Optimal flow: greedy optimization routing $\rightarrow$ **navigation efficiency**

Machine Learning (Autoencoder)

- Split both PD and control data into 120s windows (spike trains)
- Train model **ONLY** on control data $\rightarrow$ high reconstruction error leads to flagging of sample as a **'network anomaly'**



Results

Data Processing

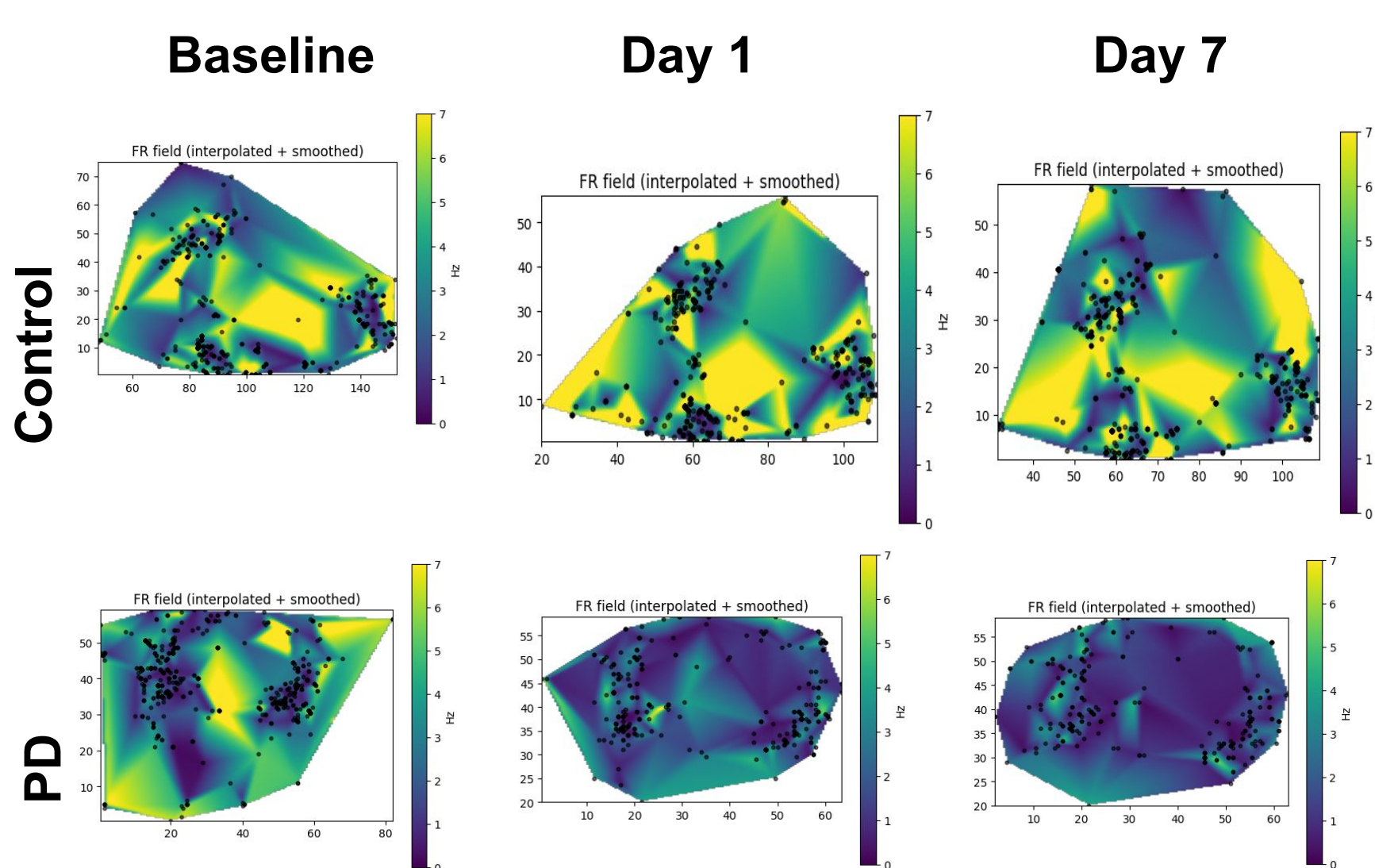


Figure 3. Spatial firing-rate maps in control vs 6-OHDA PD midbrain organoids (n=18 control, n=18 6-OHDA PD). Figure created by Andrew Shin.

Network Complexity Shift

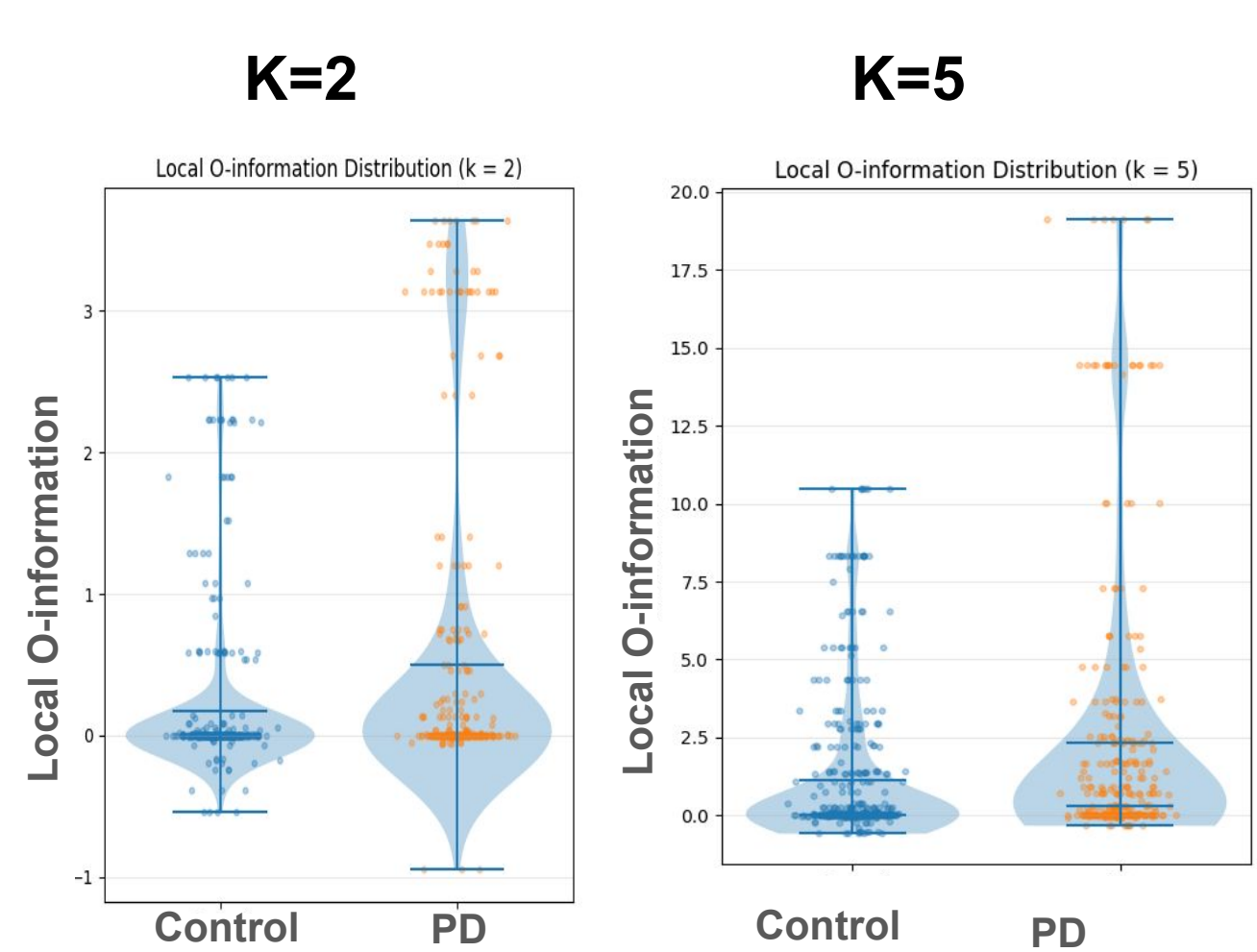


Figure 5. Local O-information distributions in control vs PD organoids at different neighborhood sizes (2 and 5). 6-OHDA PD shifts O-information toward positive values (higher redundancy), indicating reduced synergy. (k=nearest neighbors) Figure created by Andrew Shin.

Topographical Organization

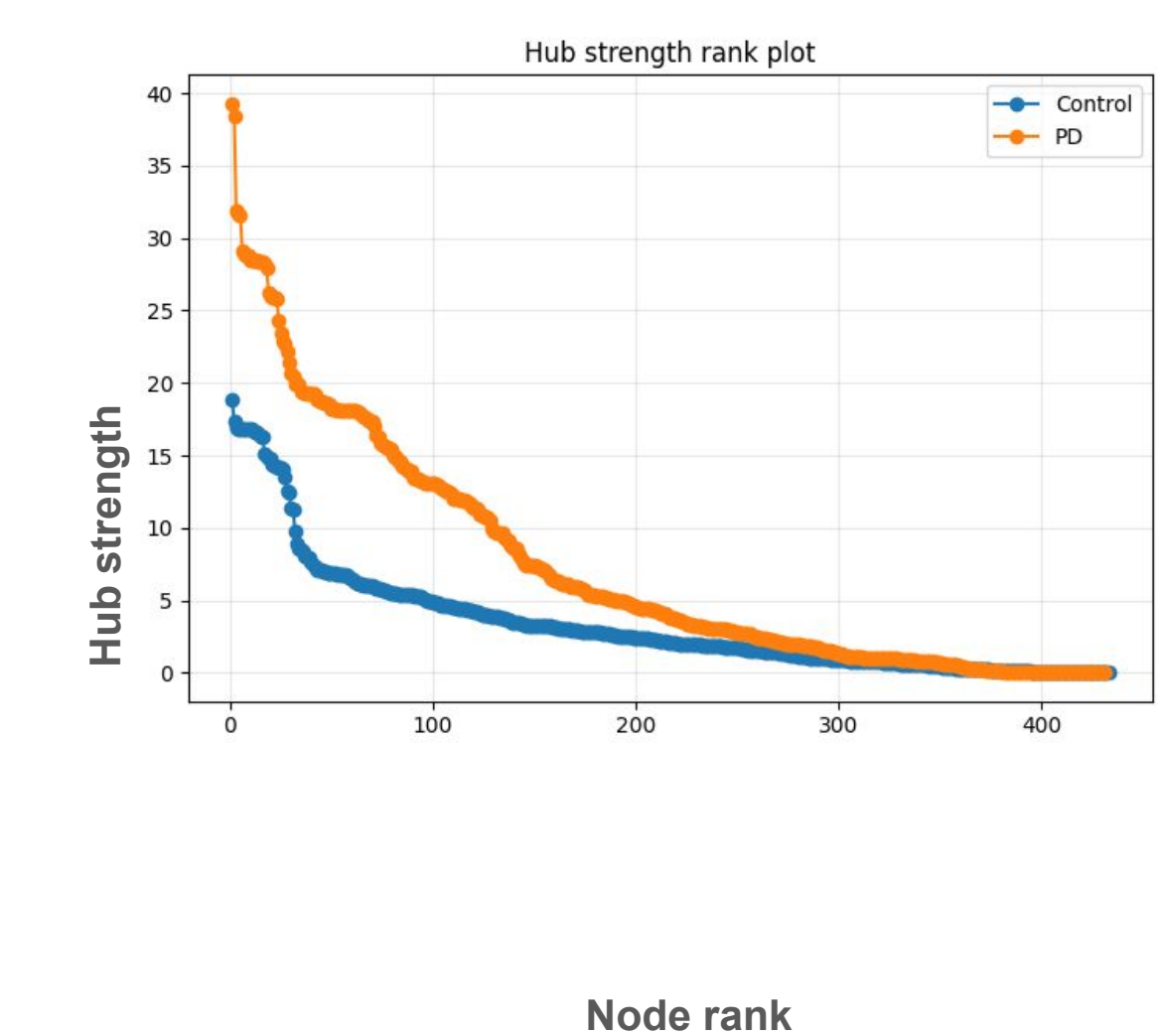


Figure 7. Hub strength rank distributions in control vs 6-OHDA PD organoid networks. 6-OHDA PD disrupts spatially organized hubs and community structure. Figure created by Andrew Shin.

Directed Information Flow

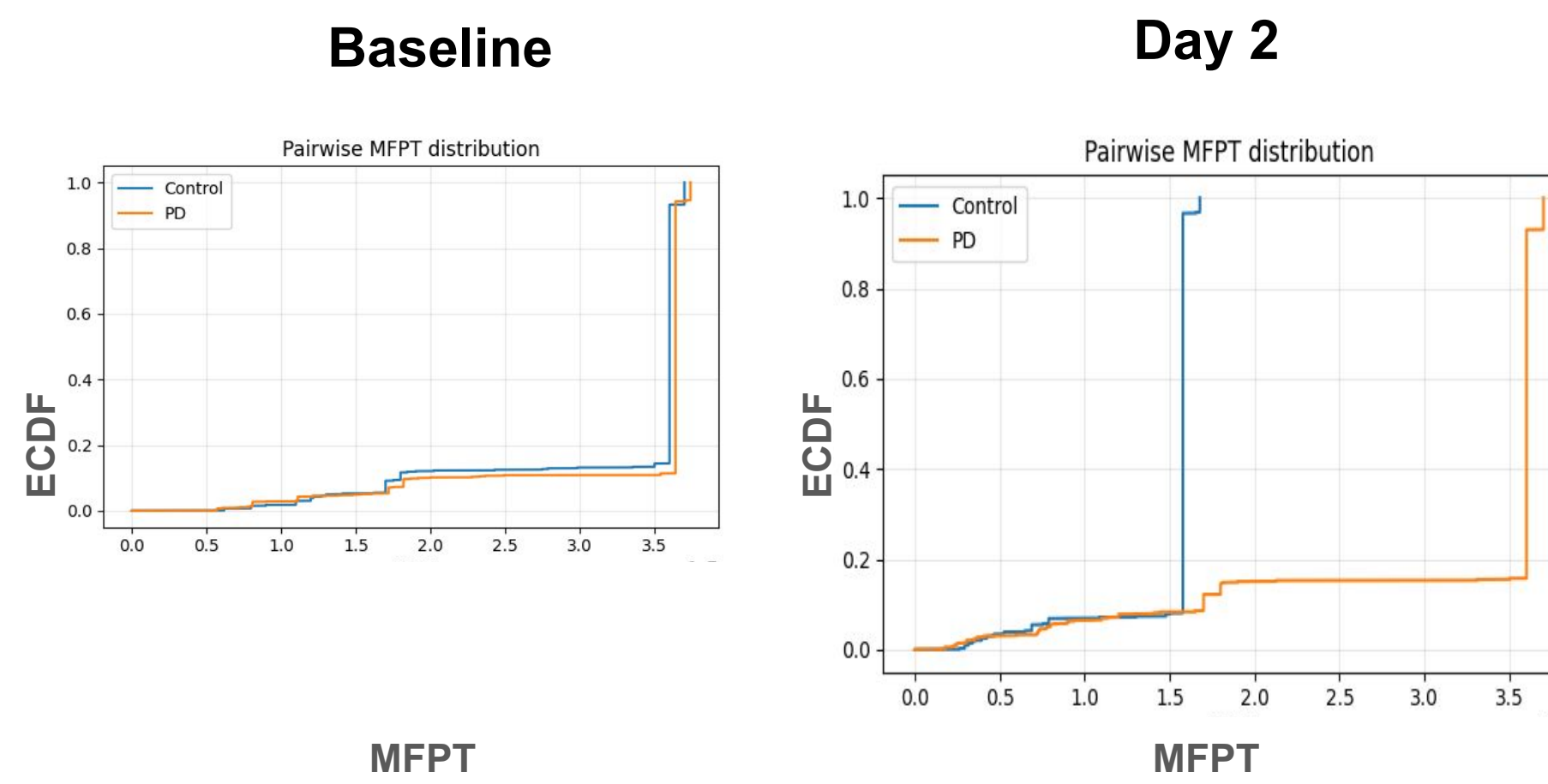


Figure 9. Pairwise Mean First-Passage Time (MFPT) distributions on Baseline and Day 2 in control vs 6-OHDA PD organoids (114 % increase in MFPT) Figure created by Andrew Shin.

Early Detectability (ML)

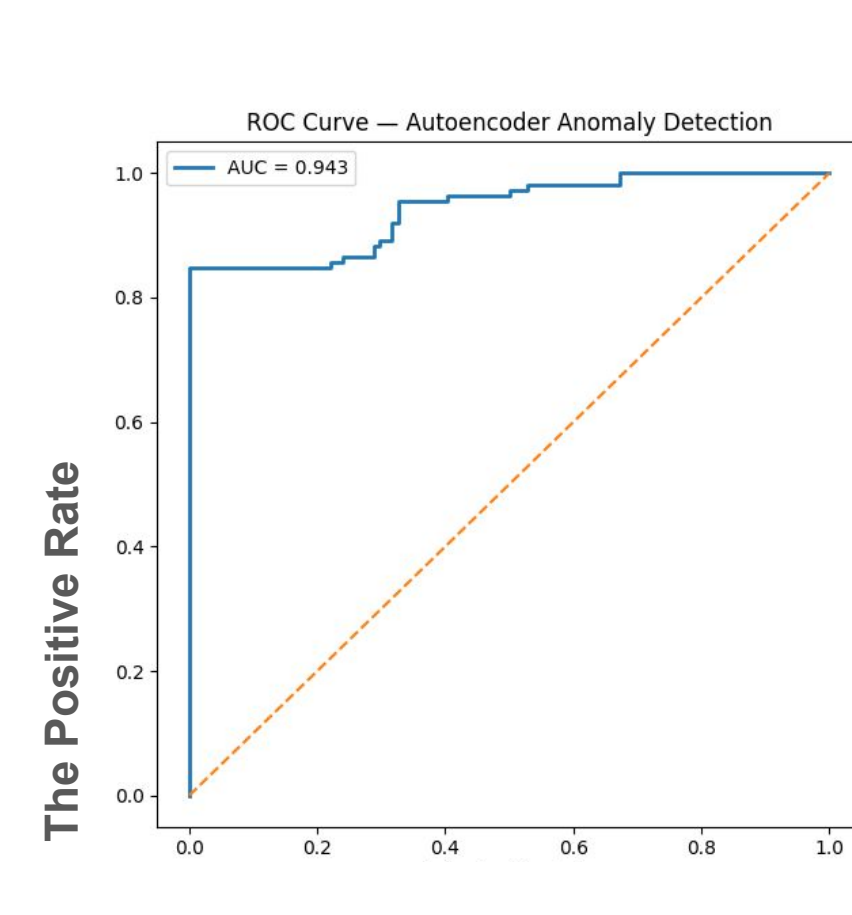


Figure 11. ROC Curve for control-trained autoencoder anomaly detection in midbrain organoid MEA recordings (**AUC=0.943**) Figure created by Andrew Shin.

Constructing Functional Graph

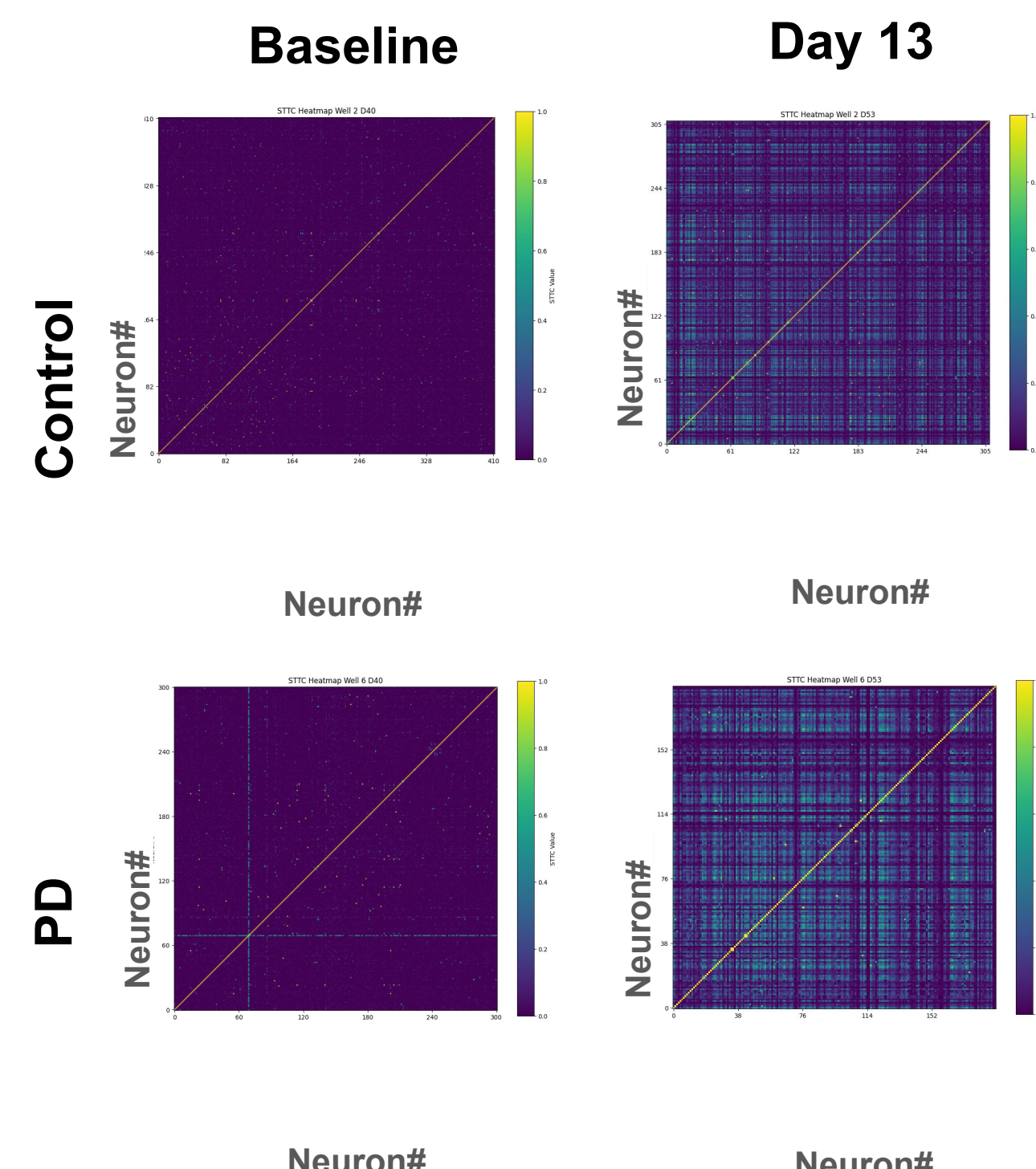


Figure 4. STTC functional connectivity heatmaps in control vs 6-OHDA PD midbrain organoids. Figure created by Andrew Shin.

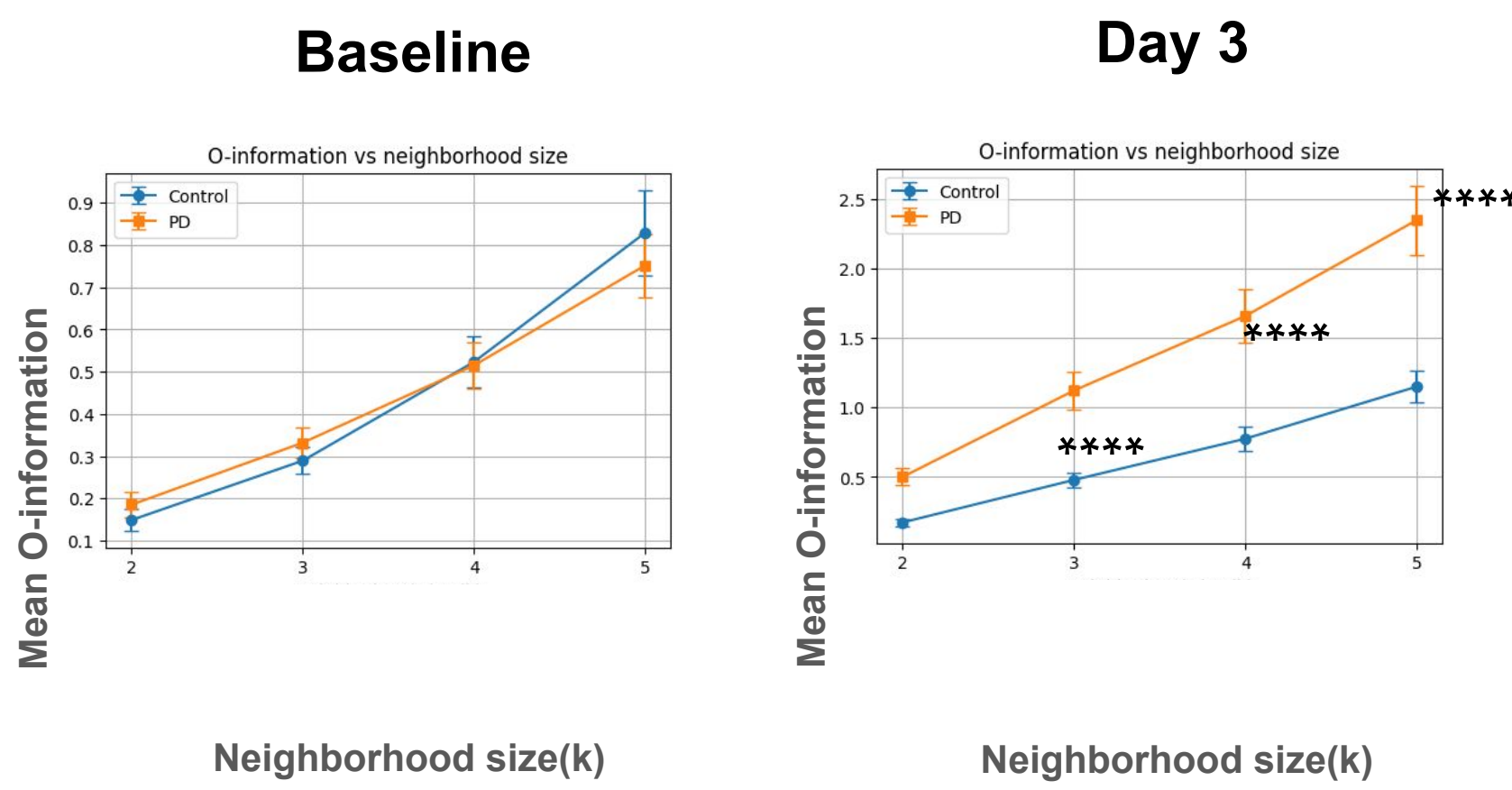


Figure 6. Longitudinal O-information across neighborhood size in control vs. PD midbrain organoids. Significance at each k was tested using a two-sided Mann-Whitney U test with FDR correction ****p<0.0001 Figure created by Andrew Shin.

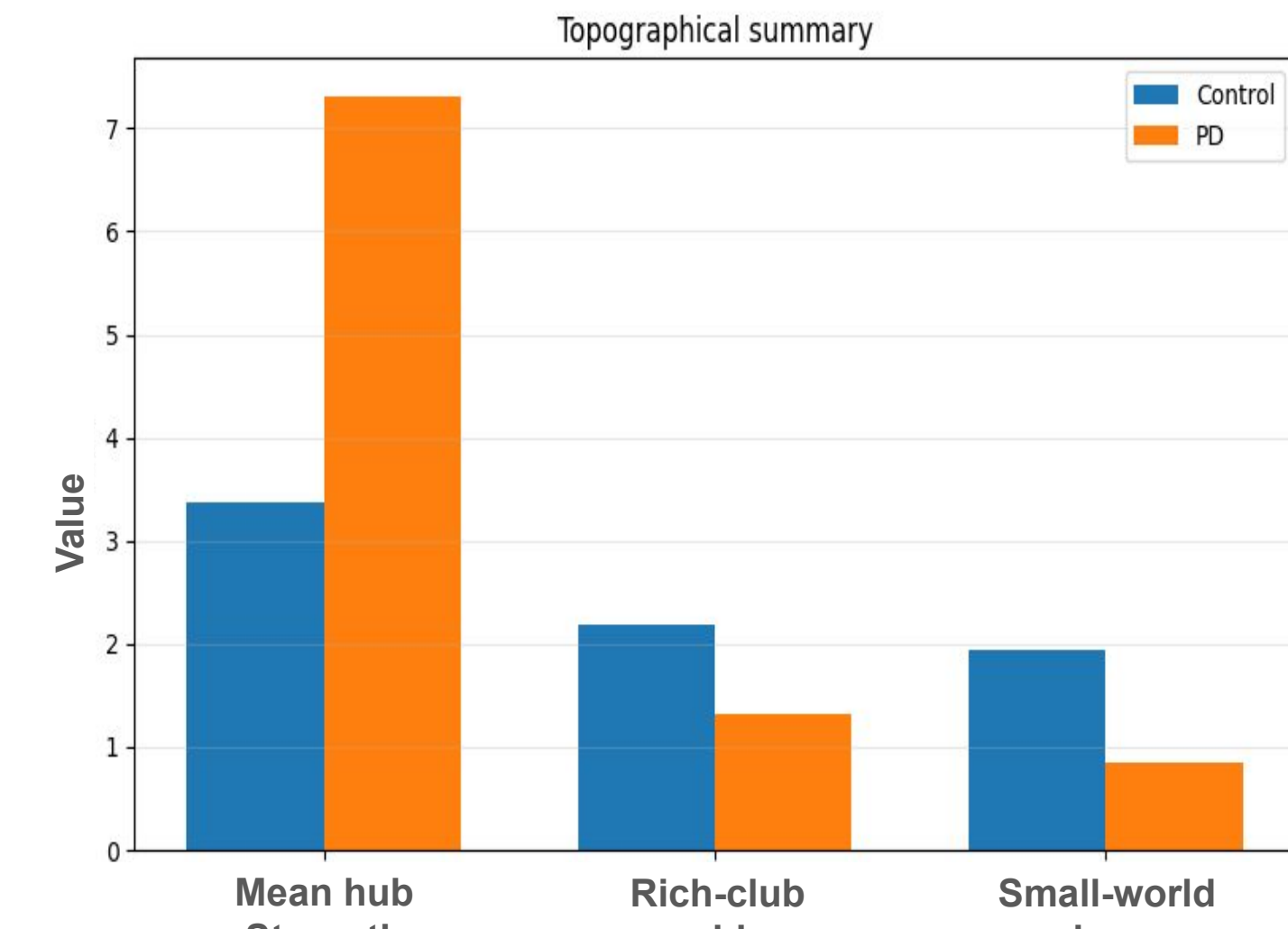


Figure 8. Topographical network summary in control vs 6-OHDA PD midbrain organoids Figure created by Andrew Shin.

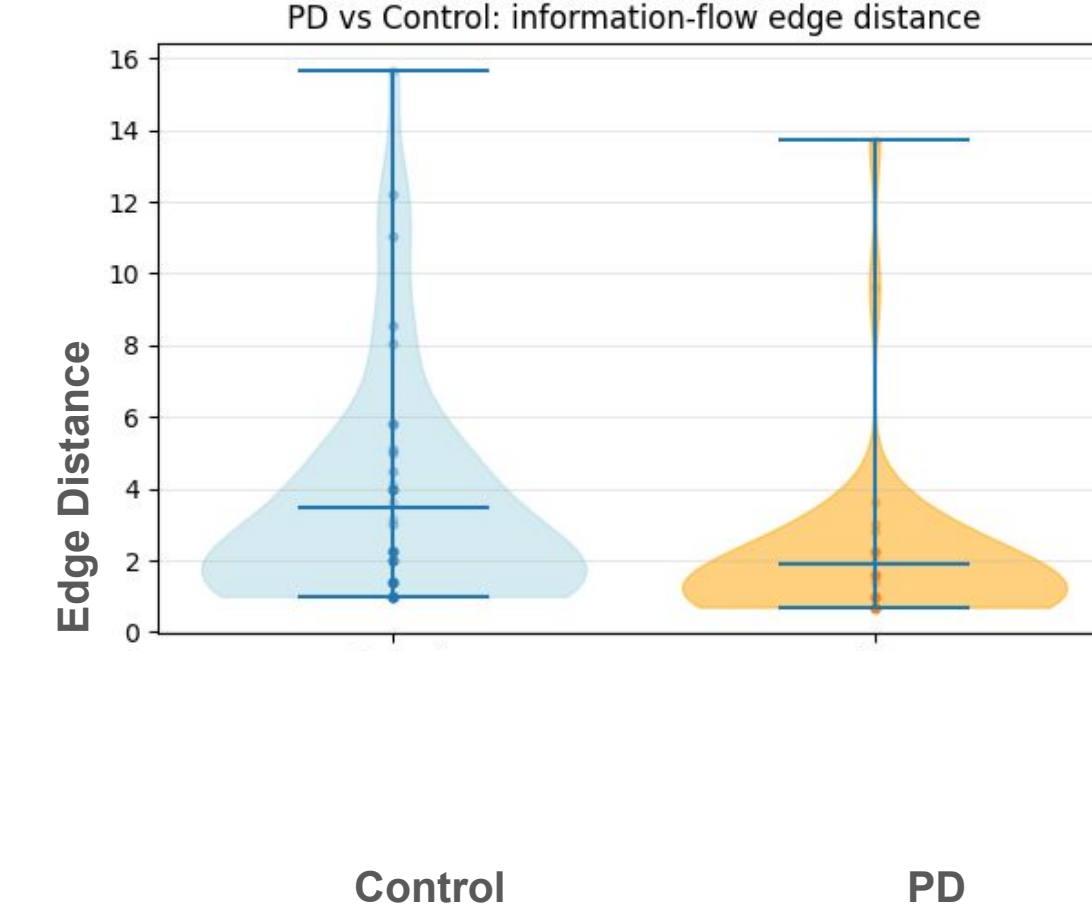


Figure 10. Spatial length of directed information-flow edges in control vs. PD organoid networks Figure created by Andrew Shin.

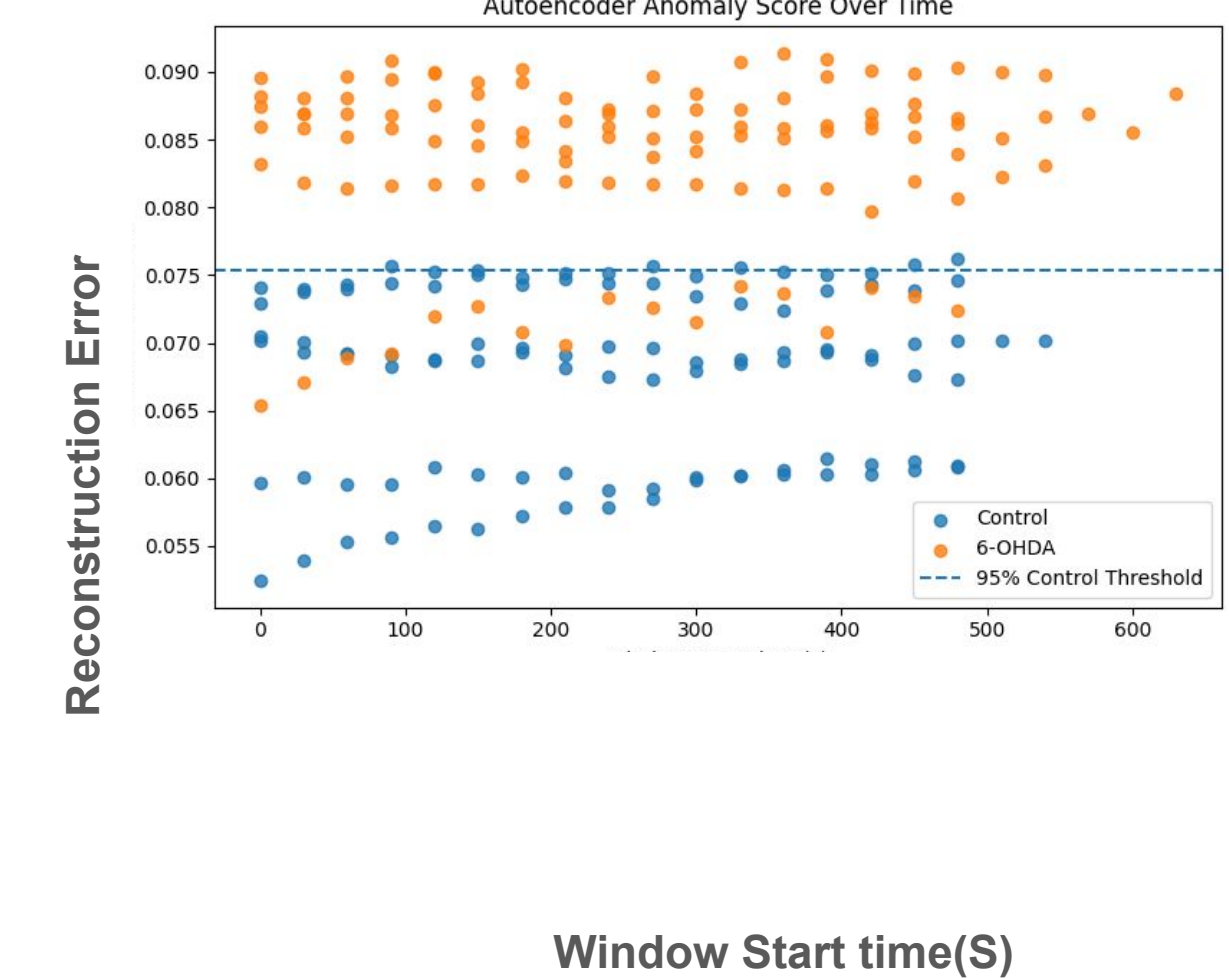


Figure 12. Autoencoder anomaly scores over time for control vs 6-OHDA PD organoid recordings Figure created by Andrew Shin.

Aim 1: PD-treated organoids show
↑ redundancy, ↓ synergy (O-info)

Aim 2: PD-treated networks show
↑ mean hub strength/
↓ rich-club / ↓ small-world

Aim 3: PD-treated organoids show
↑ MFPT, ↓ navigation efficiency (TE)

Aim 4: Autoencoder accurately
detects PD-like deviation early

Conclusion

Conclusion

1. Human midbrain organoids enable **accurate, human-relevant, 3D network-level modeling** of PD-like dysfunction.
2. Dopaminergic toxicity produces **early, quantifiable disruptions** in complexity, topology, and directed information flow.
3. Unsupervised learning detects early **PD-like dynamics**, enabling scalable electrophysiological biomarker discovery and screening.

Main Takeaways:

PD-like disruption appears as a **network-level coordination deficit** measurable from organoid electrophysiology.

Impact:

- Validates the use of brain organoids to explore symptoms/hallmarks of PD
- Produces interpretable network biomarkers beyond firing rates.
- Stronger correlation of PD as a mechanistic network disorder

Future work:

- Add dose-response and multi-toxin comparisons (rotenone, haliperidol).
- Validate signals of early degeneration via paired assays
- Extend to genetic PD organoids (LRRK2/SNCA) and test discovered signature generalization.

References:

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