

An Assembloid Model Of Frontotemporal Dementia With *MAPT* Mutations

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Background

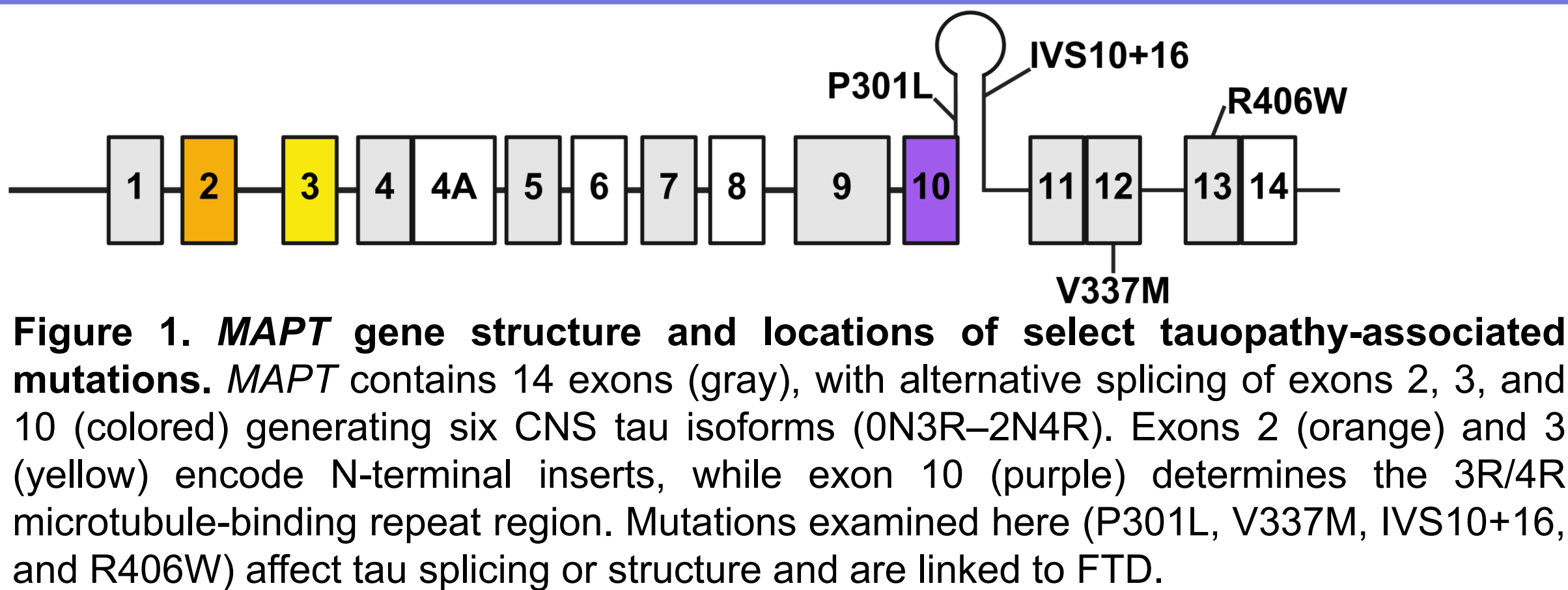


Figure 1. *MAPT* gene structure and locations of select tauopathy-associated mutations. *MAPT* contains 14 exons (gray), with alternative splicing of exons 2, 3, and 10 (colored) generating six CNS tau isoforms (0N3R–2N4R). Exons 2 (orange) and 3 (yellow) encode N-terminal inserts, while exon 10 (purple) determines the 3R/4R microtubule-binding repeat region. Mutations examined here (P301L, V337M, IVS10+16, and R406W) affect tau splicing or structure and are linked to FTD.

Frontotemporal dementia with tau pathology (FTD-tau) is a leading cause of early-onset dementia, driven by *MAPT* mutations that promote tau accumulation, neuronal dysfunction, and degeneration, particularly in the frontal and temporal cortex. **Tau pathology is also observed in the basal ganglia and substantia nigra, suggesting broader circuit involvement.** To model this, we are developing cortical-striatal-midbrain assembloids to examine how *MAPT* mutations disrupt regional interactions underlying FTD-tau progression.

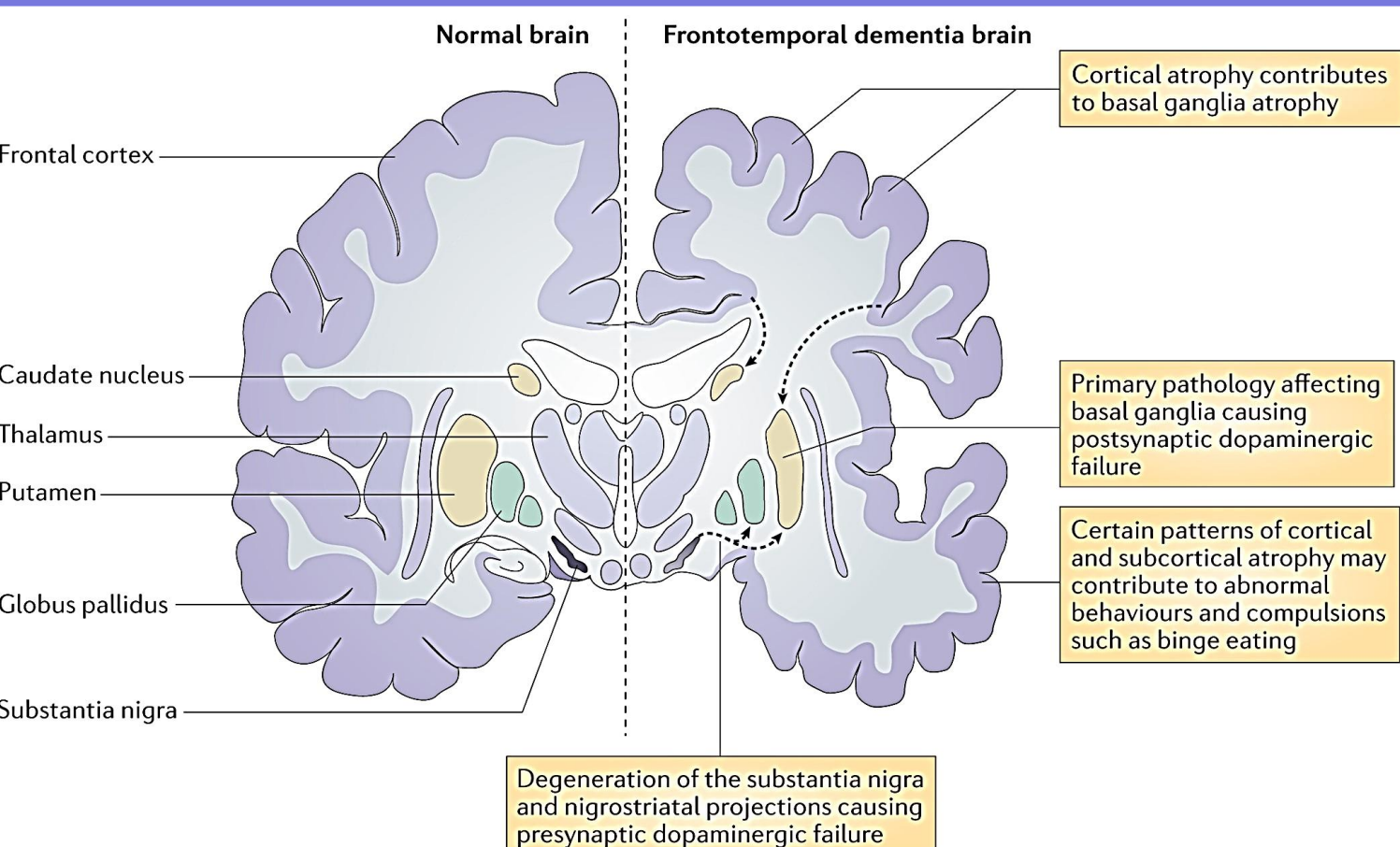


Figure 2. Cortical and subcortical involvement in frontotemporal dementia. Compared to a healthy brain (left side of figure), the FTD brain (right side of figure) shows cortical atrophy alongside degeneration of subcortical regions, including the basal ganglia and the substantia nigra, a key midbrain structure. These changes highlight vulnerability of cortico-striatal-midbrain circuits. Adapted from Baizabal-Carvallo and Jankovic, 2016.

Regionally specified brain organoids

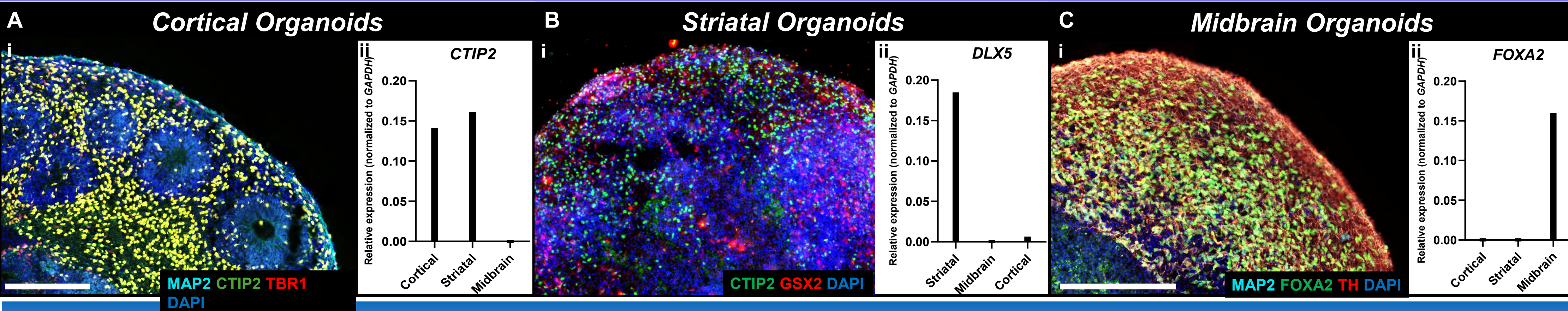


Figure 3. Region-specific identity of cortical, striatal, and midbrain organoids. (A) Cortical organoids. i) Immunohistochemistry (IHC) at 2 months shows expression of cortical neuronal markers CTIP2 and TBR1 (with MAP2 labeling neuronal populations). ii) Quantitative PCR (qPCR) at day 20 shows elevated *FOXG1* expression relative to midbrain organoids. As forebrain-derived tissues, both cortical and striatal organoids express *FOXG1*; however, cortical organoids lack expression of the striatal marker *DLX5* (see Bii). (B) Striatal organoids. i) IHC at 4 months confirms expression of the striatal marker *GSX2*. ii) qPCR at day 20 shows elevated *DLX5* expression relative to cortical and midbrain organoids. (C) Midbrain organoids. i) IHC at 2 months shows expression of midbrain floor plate and dopaminergic markers *FOXA2* and *TH* (with MAP2 labeling neuronal populations). ii) qPCR at day 20 shows elevated *FOXA2* expression relative to cortical and striatal organoids. For qPCR, 3 organoids were pooled per sample/region. Scale bar: 200 um.

Model Development

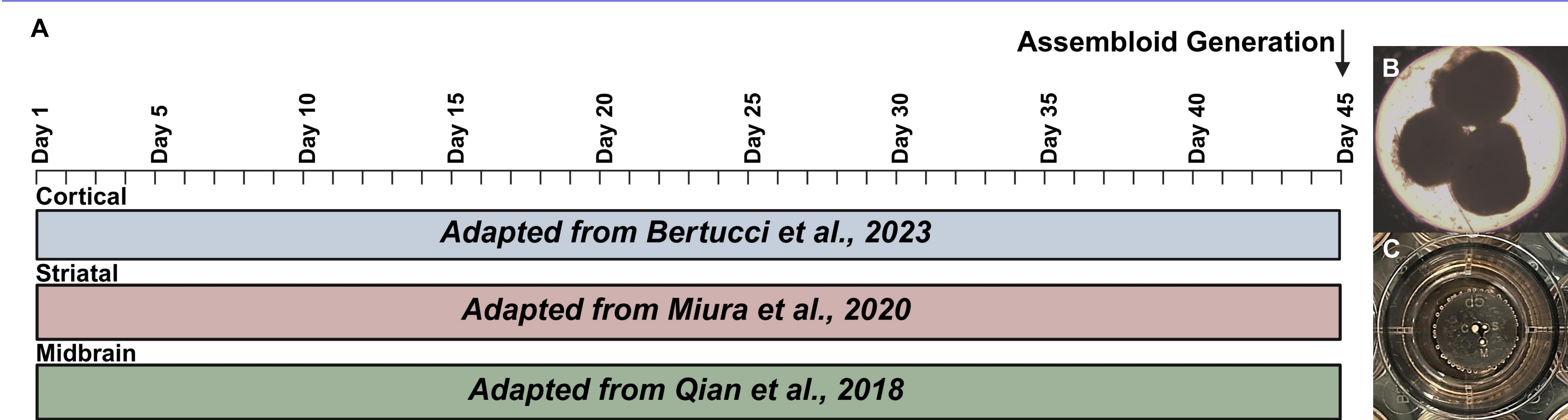


Figure 4. Regional brain organoid differentiation and assembloid generation strategies. (A) Region-specific brain organoids are generated independently using established protocols, with distinct media formulations supporting cortical, striatal, and midbrain identities. (B–C) Two complementary approaches are used to generate cortical-striatal-midbrain assembloids. (B) Version 1: Organoids from each region are combined in a single well of a 96-well plate to allow spontaneous assembly. (C) Version 2: Organoids are positioned within a laser-cut acrylic mold that maintains regional separation while promoting directed axonal growth through connecting channels. The mold is mounted on a 16 mm glass coverslip using adhesive backing, and the culture surface is coated with Cultrex or laminin prior to assembly to support organoid attachment.

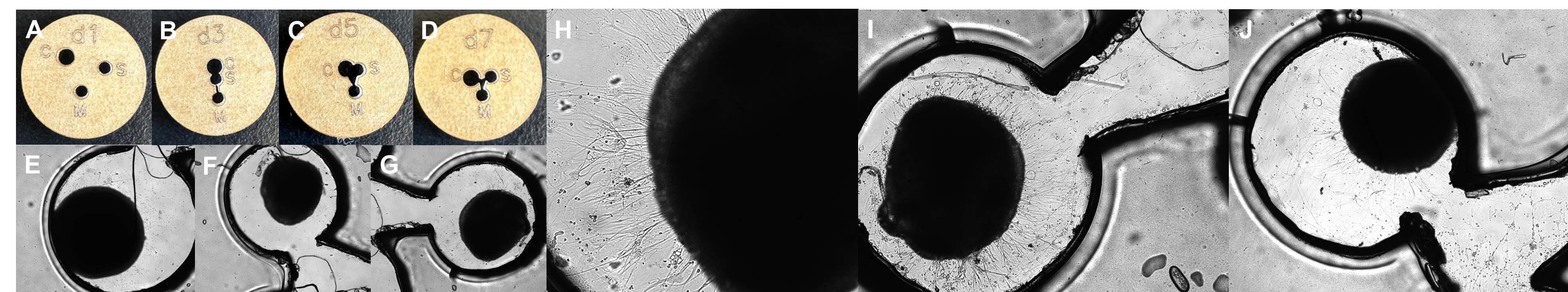


Figure 5. Version 2 assembloids support directed axonal growth between organoid regions. (A–D) Laser-cut acrylic molds. One control design (A) and three experimental designs (B–D) were evaluated to optimize inter-regional axonal growth. (E–H) Organoids plated on laminin-coated molds exhibit robust axonal outgrowth. Molds were mounted onto 16 mm glass coverslips prior to laminin coating and plating of cortical (E) and midbrain (F–H) organoids. Axonal projections extending from individual organoids can be observed after one week in culture. (H) After one month, axonal projections from one midbrain organoid plated on laminin extend into the channels connecting adjacent organoid regions. (I–J) After one month in culture, axonal projections from midbrain organoids extend into the channels connecting adjacent organoid regions in laminin-coated (I) and Cultrex-coated (J) molds.

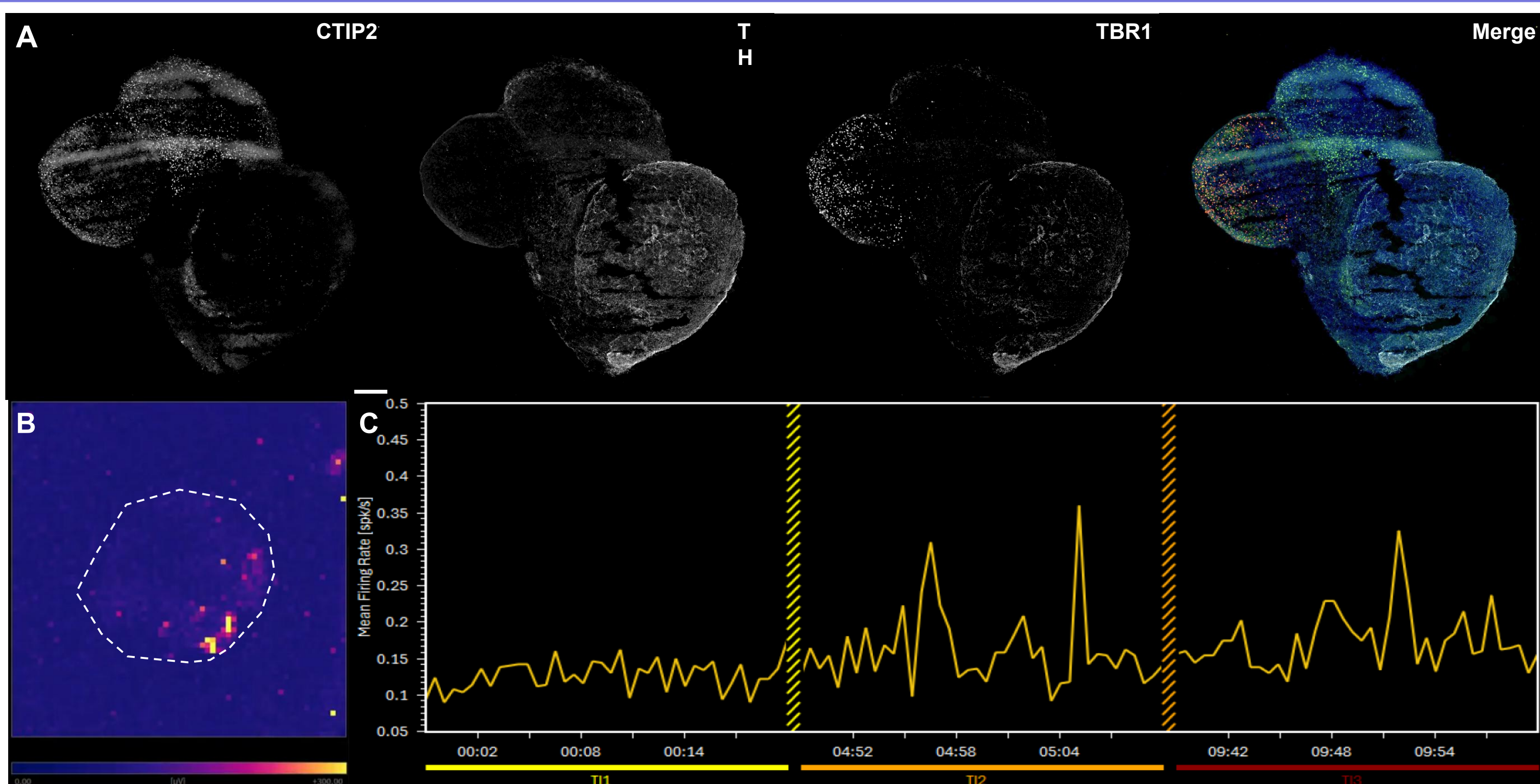


Figure 6. Version 1 assembloids exhibit region-specific identity and electrophysiological activity. (A) WT assembloids were harvested after ~16 days of assembly (total organoid age: 2 months). IHC shows spatially restricted expression of the cortical/striatal marker CTIP2, cortical marker TBR1, and midbrain dopaminergic marker TH across distinct assembloid regions. (B) Network activity was assessed using 3Brain HyperCAM Alpha or BioCAM Duplex systems. Localized electrophysiological activity was detected in regions of assembloid-electrode contact and is displayed as peak-to-peak amplitude. (C) Mean firing rate across a 10-minute recording increased over sequential recording intervals (T11–T13). Scale bar: 200 um.

Future Directions

- Incorporation of microglia into assembloid models
- Integration of mold-based assembloids with 3Brain MEA platforms; calcium imaging as an additional functional readout
- Comparison of assembloid profiles to developing and mature human brain regions
- Generation of assembloids from *MAPT* mutant isogenic pairs (V337M, R406W, P301L, IVS10+16)

Acknowledgements

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