Closed-loop stimulation reveals learning-related plasticity in paired human brain organoids

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Introduction

Learning and memory rely on activity-dependent neural plasticity, yet human-relevant in vitro models capable of measuring persistent functional changes remain limited. This represents a significant challenge for investigating neurological disorders and evaluating therapeutic interventions targeting cognitive dysfunction. We developed a multiwell human brain organoid platform integrating chronic electrophysiological recording with open-loop and closed-loop stimulation paradigms. Using this system, we investigated persistent network potentiation, adaptive task performance, and associated transcriptional changes to establish complementary functional measures of learning-related plasticity.

Human Brain Organoid Connectoid Platform

Human iPSC-derived CNS-3D cortical organoids[1] were cultured in pairs within custom 24-well embedded electrode array (EEA) plates[2]. Guided axonal outgrowth formed interconnected neural networks, termed connectoids, across ten integrated microelectrodes. Continuous inter-organoid connections developed over 5 weeks across separation distances of 9 mm. The resulting platform supports longitudinal measurement of spontaneous activity, electrically evoked responses, and network adaptation under controlled experimental conditions.

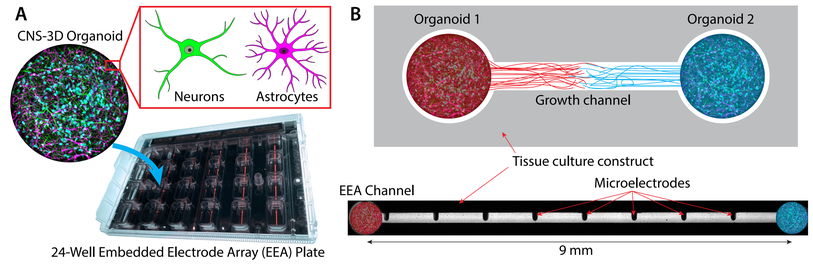


Figure 1. Human cortical organoids and the connectoid electrophysiology platform. (A) Representative CNS-3D organoid stained for neurons (MAP2, green) and astrocytes (GFAP, magenta), alongside the custom 24-well EEA plate. (B) Schematic of paired organoids connected through a guided axonal growth channel containing ten embedded microelectrodes. The illustrated configuration features a 9 mm separation between organoids.

Repeated Stimulation Induces Network Potentiation

To investigate activity-dependent plasticity, connectoids underwent daily open-loop evaluations with or without additional electrical stimulation. Evoked spike counts were tracked to measure changes from baseline. Repeated stimulation produced larger responses over successive days, with enhanced magnitude and persistence following BDNF supplementation (50 ng/mL). NMDA receptor blockade with AP5 (200 µM) prevented potentiation, supporting the involvement of plasticity-related signaling pathways.

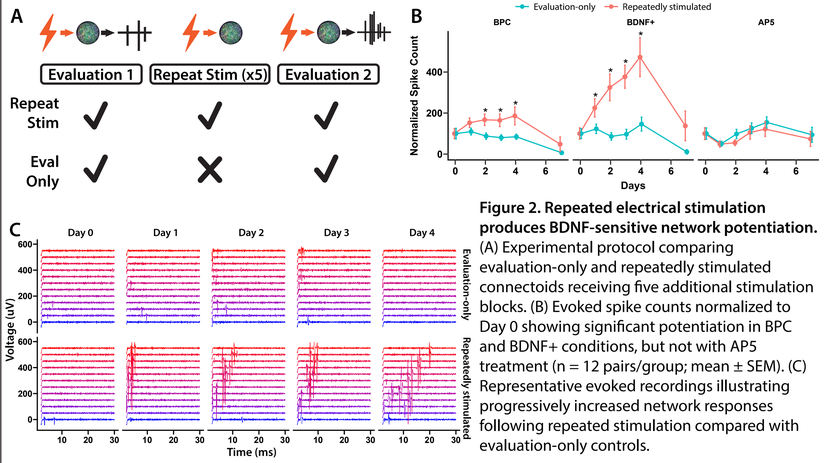


Figure 2. Repeated electrical stimulation produces BDNF-sensitive network potentiation. (A) Experimental protocol comparing evaluation-only and repeatedly stimulated connectoids receiving five additional stimulation blocks. (B) Evoked spike counts normalized to Day 0 showing significant potentiation in BPC and BDNF+ conditions, but not with AP5 treatment (n = 12 pairs/group; mean ± SEM). (C) Representative evoked recordings illustrating progressively increased network responses following repeated stimulation compared with evaluation-only controls.

Closed-loop Maze Gameplay

To evaluate adaptive network behavior, connectoids were interfaced with a closed-loop Pac-Man-like virtual maze. Directional electrical stimuli encoded food and danger cues, while evoked spike responses determined movement in real time. Training incorporated outcome-dependent feedback, with food capture triggering a stimulation pause and danger encounters triggering electrical penalties. A five-pulse, 100 Hz penalty pattern produced progressive improvements in points scored and survival time compared with evaluation-only controls. These training-associated performance gains were absent following BDNF depletion, indicating sensitivity to plasticity-related signaling.

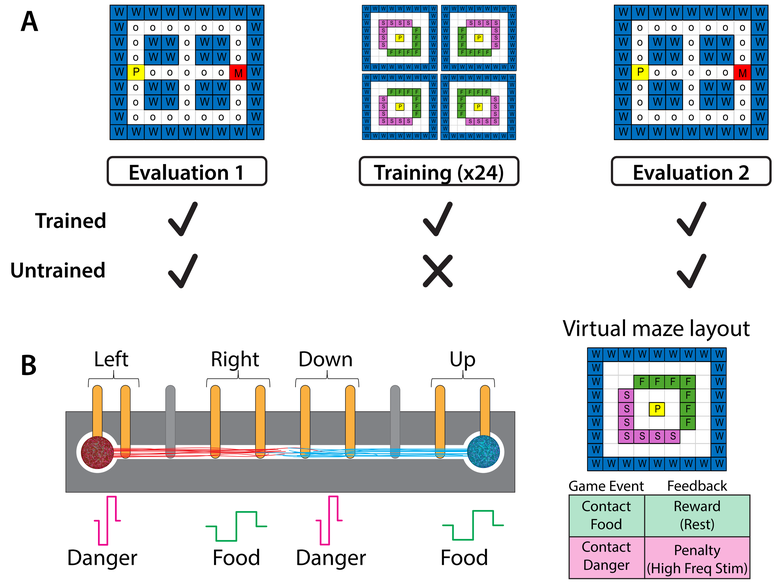


Figure 3. Closed-loop maze training promotes BDNF-dependent improvements in task performance. (A) Daily training paradigm consisting of evaluations before and after 24 feedback-training trials, compared with evaluation-only controls. (B) Schematic of directional electrode mapping and virtual maze inputs, where neural responses control movement and task outcomes determine subsequent feedback. (C) Normalized survival time across training days in standard BPC and BDNF-depleted media (n = 9 organoid pairs/group; mean ± SEM). Feedback-trained connectoids showed significantly improved survival in standard medium, while BDNF removal eliminated the training-associated advantage.

Transcriptomic Analysis of Neural Plasticity

Bulk RNA sequencing was used to investigate molecular changes associated with network potentiation and closed-loop training. Gene-set variation analysis (GSVA) revealed elevated learning, memory, and synaptic plasticity signatures following repeated stimulation and maze training. These signatures were enhanced by BDNF supplementation but reduced by AP5 treatment or BDNF depletion, consistent with the observed functional outcomes.

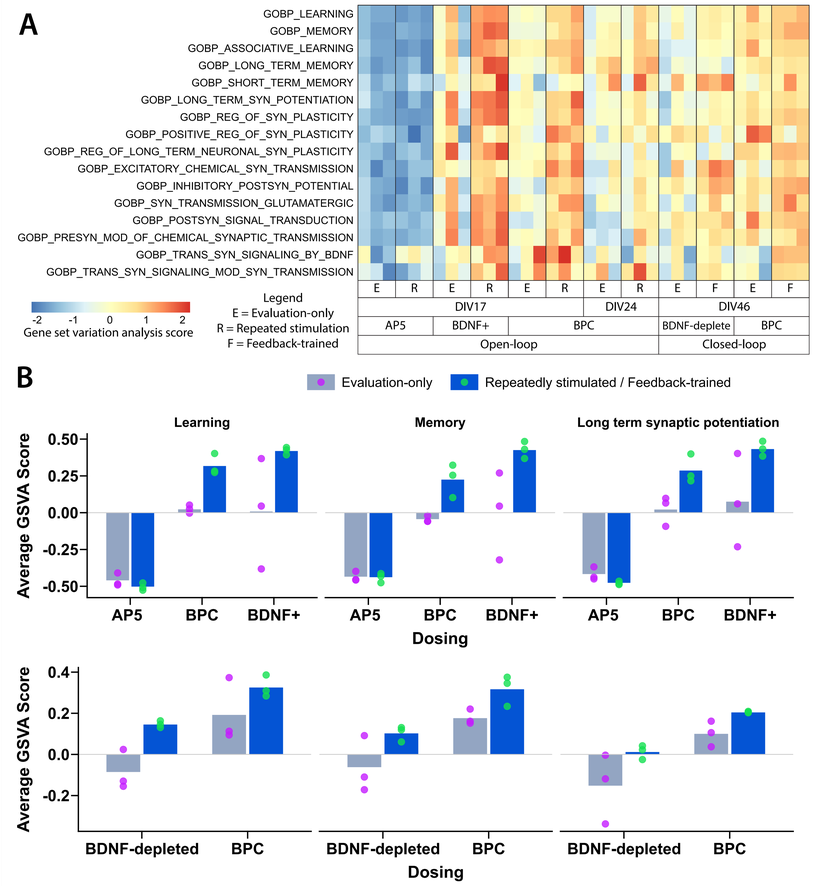


Figure 4. Transcriptomic signatures of learning-related plasticity. (A) GSVA heat map of 15 learning, memory, and synaptic plasticity gene sets across experimental conditions. (B) Summary GSVA scores for network potentiation (top) and closed-loop maze training (bottom), showing enhanced plasticity-related signatures following training and reduced scores with AP5 or BDNF depletion (n = 3 pooled biological replicates/condition).

Conclusions & Future Directions

Our human brain organoid connectoid platform enables longitudinal investigation of persistent network potentiation and adaptive task performance. The sensitivity of these responses to BDNF availability and NMDA receptor blockade, together with corresponding transcriptomic changes, supports their relevance to learning-related neural plasticity.

Future studies will expand the biological complexity and translational applications of the platform through:

- Incorporation of additional cell populations, including microglia and oligodendrocytes.
- Evaluation of disease-associated plasticity deficits, including Alzheimer's disease models.
- Development of functional screening assays to investigate therapeutic modulation of learning and memory-related dysfunction.

1. Marchetto, M. C. N. et al. A model for neural development and treatment of Rett syndrome using human induced pluripotent stem cells. Cell 143, 527–539 (2010).
2. Rountree, C. et al. Human Peripheral Nerve-on-a-Chip on a Multiwell Microelectrode Array as a Scalable Preclinical Neurotoxicity Assay. 2025.06.09.658615 Preprint at https://doi.org/10.1101/2025.06.09.658615 (2025).

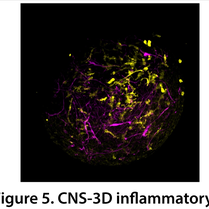


Figure 5. CNS-3D inflammatory with microglia (yellow; Iba1) and astrocytes (magenta; GFAP)