

Supplementary information for

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Methods

Live/Dead staining

The viability of cells in the NA models was assessed on DIV1 and DIV7 using the LIVE/DEAD® Viability/Cytotoxicity Kit (L3224, Thermo, USA). Live cells are marked with green fluorescence and dead cells are marked with red fluorescence. Fluorescence images of the cells were obtained using a laser scanning confocal microscope (LSCM; A1, Nikon, Japan). ImageJ software was used to count live and dead cells. Cell viability was quantified as the ratio of green cells to the total number of cells ($n=3$).

Neurite density analysis

To evaluate neurite density in the NA models, immunofluorescence images stained for MAP2 were analyzed using ImageJ. To minimize the influence of differences in fiber occupancy among experimental groups on neurite density quantification, only the non-fiber regions of each group were included in the analysis. Because the fiber regions were identifiable in the blue channel but were negative for MAP2 staining, these fiber-occupied regions were first identified and excluded from the subsequent analysis. Large soma-like MAP2-positive regions were excluded as much as possible according to their size and morphology, and the remaining neurite-associated MAP2-positive signals were used for quantification. Neurite density was calculated as: $\text{Neurite density (\%)} = (\text{Area of neurite-associated MAP2-positive signals} / \text{Area of non-fiber region}) \times 100\%$. $n=5$ independent fields were analyzed for each group.

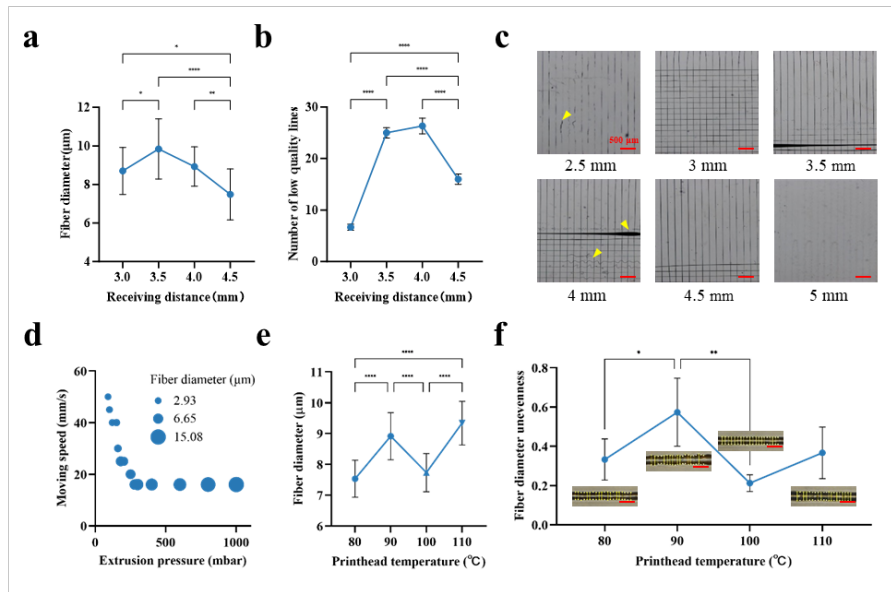


Fig. S1 Optimization of fiber printing parameters. (a) Effect of receiving distance on fiber diameter. (b) Effect of receiving distance on the number of low-quality lines. (c) Representative fiber morphologies at different receiving distances (scale bar: 500 μm). (d) Combined effects of extrusion pressure and moving speed on fiber diameter. (e) Effect of nozzle temperature on fiber diameter. (f) Effect of nozzle temperature on fiber diameter uniformity. Data are presented as mean±SD ($n \geq 3$); statistical significance is denoted as * $p < 0.05$, ** $p < 0.01$, and **** $p < 0.0001$.

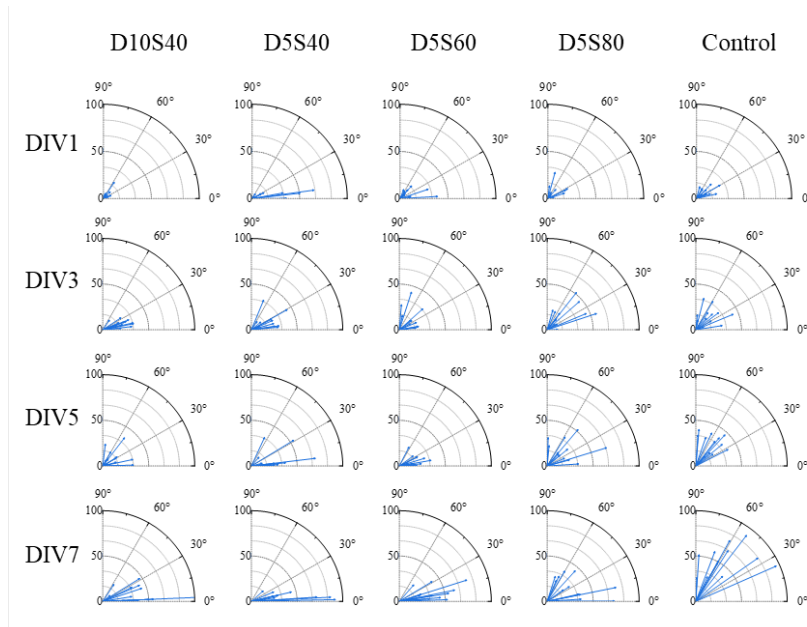


Fig. S2 Radar plots of neurite orientation in neuronal alignment models. Radar charts showing neurite length and angle relative to fiber direction for neurons in different fiber configurations at DIV1, DIV3, DIV5, and DIV7 ($n \geq 14$).

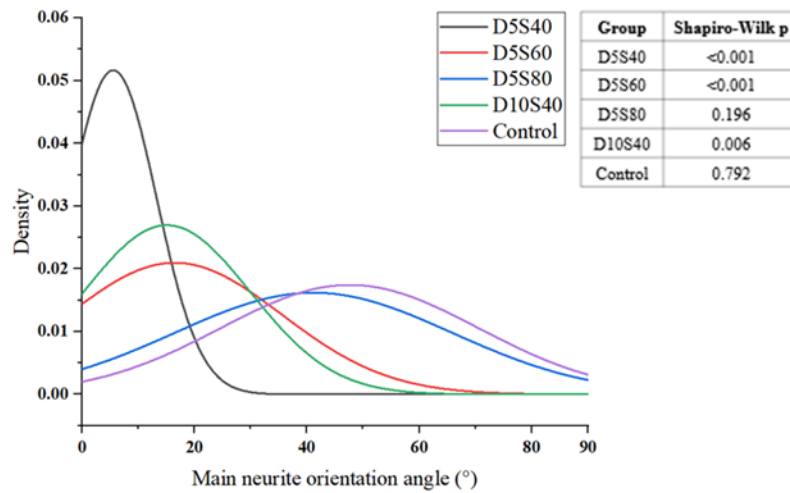


Fig. S3 Fitted normal distribution curves of main neurite orientation angles. The table summarizes the results of the Shapiro–Wilk normality test for each group.

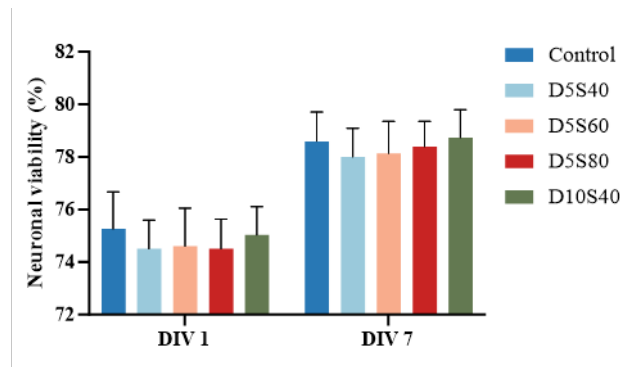


Fig. S4 Neuronal viability analysis in the NA model with different fiber structural parameters. Data are presented as mean±SD ($n=3$).

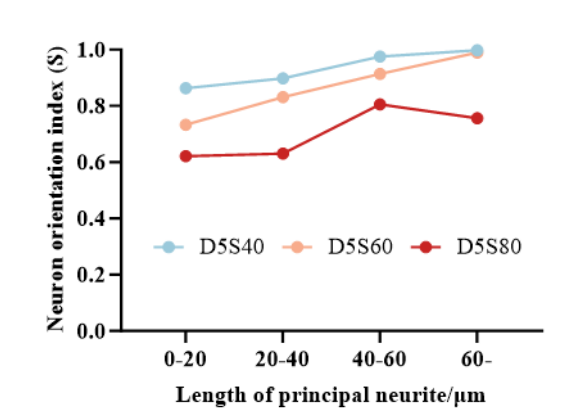


Fig. S5 Correlation between neurite length and alignment. Line chart showing the relationship between principal neurite length and neuronal orientation rate in NA models with different fiber spacings.

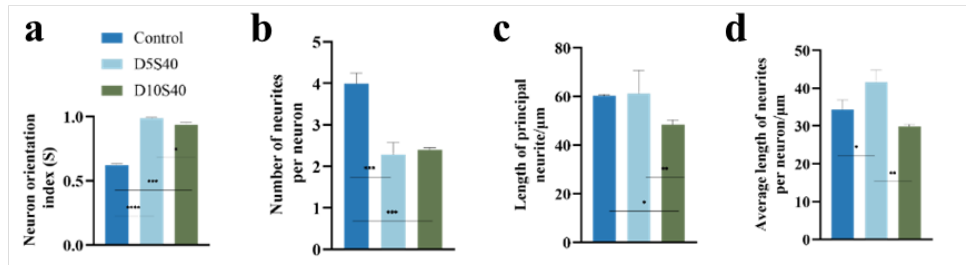


Fig. S6 Effect of fiber diameter on neuronal morphology at DIV7. Quantitative analysis of (a) orientation index (S), (b) neurite number, (c) principal neurite length, and (d) average neurite length across NA models with different fiber diameters. Data are mean $\pm$ SD ($n\geq 3$); * $p<0.05$, ** $p<0.01$, *** $p<0.001$, **** $p<0.0001$.

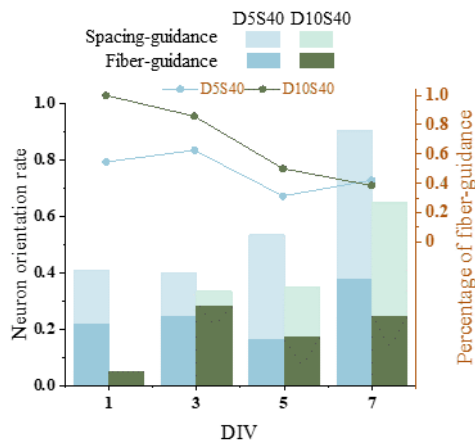


Fig. S7 Proportions of fiber-guided vs. spacing-guided neurons across fiber diameters. Stacked bar charts (black, left y-axis) show proportions of fiber-guided (dark lower segments) and spacing-guided (light upper segments) neurons at DIV1, DIV3, DIV5, and DIV7. Line plots (gold, right y-axis) represent the proportion of fiber-guided neurons relative to the total oriented population across different diameters.

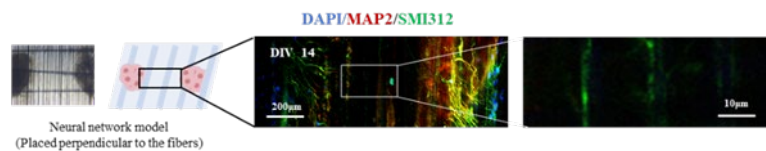


Fig. S8 Immunofluorescence staining of the central region of the control model with the two neural tissues placed perpendicular to the fiber orientation at DIV14 (nuclei, DAPI, blue; dendrites, MAP2, red; axons, SMI312, green).

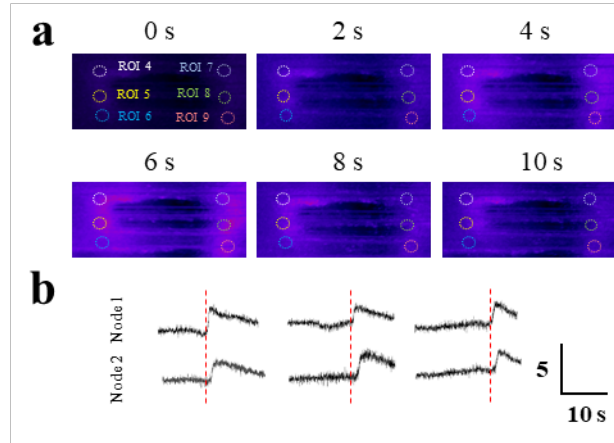


Fig. S9 Representative calcium signal transmission between paired neural tissues in the two-node neural network model. Shown are three additional examples of fluorescence intensity traces, corresponding to independent node pairs beyond those presented in Figs. 6d and 6e. Electrical stimulation of Node 1 induced a distinct calcium peak in Node 1, followed by a delayed peak in Node 2, confirming reproducible inter-tissue calcium signaling across multiple replicates.

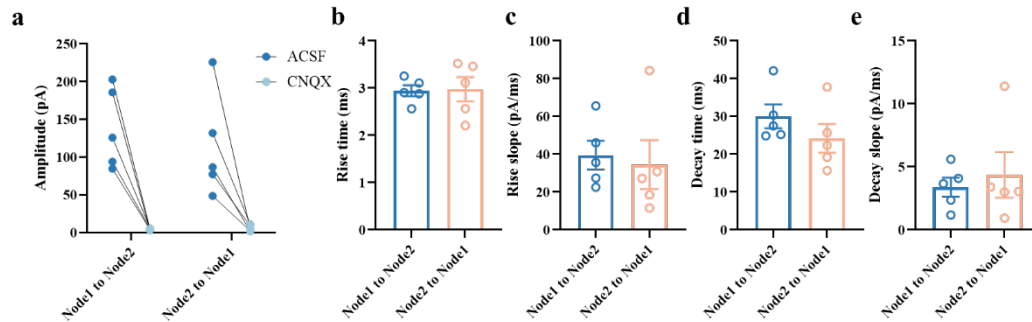


Fig. S10 CNQX sensitivity, and synaptic event statistics of evoked responses in the NN model. (a) Comparison of evoked response amplitudes recorded in the NN model under ACSF and CNQX conditions. (b–e) Rise time, rise slope, decay time, and decay slope of the evoked responses recorded in the NN model. Data are presented as mean ± SD ($n=5$).

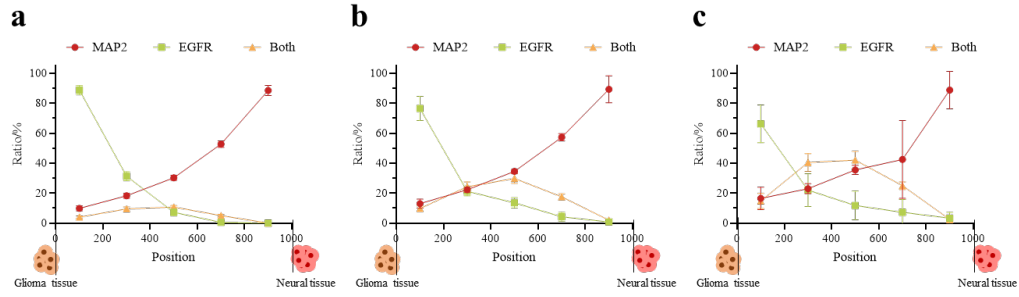


Fig. S11 Changes in the proportions of MAP2-labeled cells, EGFR-labeled cells, and double-positive events along the GBM–neuron axis at DIV4 (a), DIV7 (b), and DIV10 (c). Data are presented as mean $\pm$ SD ($n=4$).

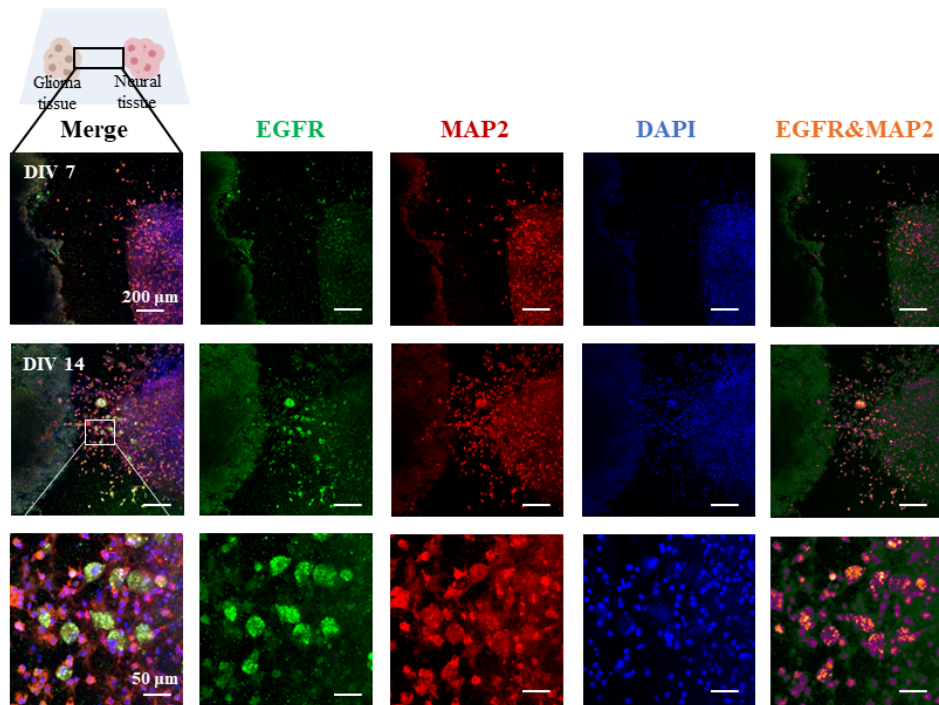


Fig. S12 Structural interactions between glioma and neural tissues in the isotropic control model without fiber guidance. Immunofluorescence images at DIV7 and DIV14 showing glioma cells (EGFR⁺, green), neurons (MAP2⁺, red), and nuclei (DAPI, blue). Merged images highlight limited cell dispersion and interface mixing in the absence of aligned fibers. Scale bars: 200 μ m (overview), 50 μ m (magnified view). The schematic above illustrates the experimental setup.

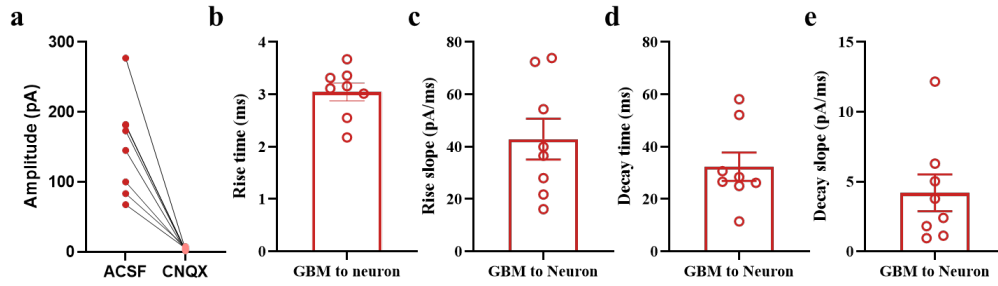


Fig. S13 CNQX sensitivity, and synaptic event statistics of evoked responses in the GN model. (a) Comparison of evoked response amplitudes recorded in the GN model under ACSF and CNQX conditions. (b–e) Rise time, rise slope, decay time, and decay slope of the evoked responses recorded in the GN model. Data are presented as mean±SD ($n=8$).

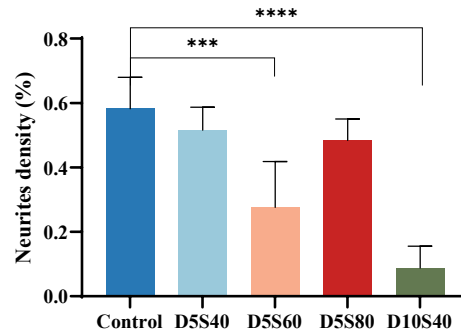


Fig. S14 Neurite density analysis in the NA model with different fiber structural parameters. Data are presented as mean±SD ($n=5$).

Table S1. Summary of patient-derived GBM samples used in the GN model

Patient ID	P1	P2	P3
Tumor location	Left frontal lobe	Right frontal lobe	Left frontoparietal
Diagnosis	GBM, CNS WHO grade 4	GBM, CNS WHO grade 4	GBM, CNS WHO grade 4
IDH1/IDH2	Mutation not detected	Mutation not detected	Mutation not detected
TERT promoter	C250T	C250T	C250T
MGMT	IHC-; MGMT promoter methylation NA	IHC-; MGMT promoter methylation NA	NA
Ki-67	~40%	~50%	~40%
p53 pattern	Wildtype-like	Mutant-like	Wildtype-like
c-MET	~30%	~20%	Negative

Table S2. Patient usage across assays

Patient ID	GN morphology (DIV7/14)	GN electrophysiology (DIV14)
P1	√	
P2		√
P3		√

Table S3. Antibodies used for immunofluorescence staining

Antibody	Source (Company, Catalog)	Secondary antibody (Company, Catalog)	Application
MAP2	Proteintech, 17490-1-AP	Goat anti-rabbit Alexa Fluor 594 (Proteintech, SA00013-4) / Goat anti-rabbit Alexa Fluor 488 (Proteintech, SA00013-1)	Neuronal morphology
SMI312	Biologend, 837904	Goat anti-mouse Alexa Fluor 488 (Proteintech, SA00013-1)	Neuronal morphology
PSD95	Abcam, ab192757	Goat anti-mouse Alexa Fluor 488 (Proteintech, SA00013-1)	Synapse staining
SYN1	Abclonal, A5247	Goat anti-rabbit Alexa Fluor 594 (Proteintech, SA00013-4)	Synapse staining
GFAP	Sigma, AB5541	Goat anti-chicken Alexa Fluor 568 (Abcam, ab175477)	Glial cell type
EGFR	Immunoway, YM4442	Goat anti-mouse Alexa Fluor 488 (Proteintech, SA00013-1)	Glioma cell type