[Article]

Microfluidics-Guided Localized Low-Temperature Modulation of Axonal Signal Propagation

Jaehyun Kim[a], Eunseok Seo[a], Na Yeon Kim[a], Bong Geun Chung[a], Jungchul Lee[b], Taesung Kim[c], Seung-Woo Cho[d,e], Gunho Kim[*c], Sung Soo Kim[*f] and Jungyul Park[*a,g]

[a]*Department of Mechanical Engineering, Sogang University, 35 Baekbeom-ro, Mapo-gu, Seoul 04107, Republic of Korea.*

[b]*Department of Mechanical Engineering, Korea Advanced Institute of Science and Technology (KAIST), Daejeon 34141, Republic of Korea*

[c]*Department of Mechanical Engineering, Ulsan National Institute of Science and Technology (UNIST), Ulsan 44919, Republic of Korea*

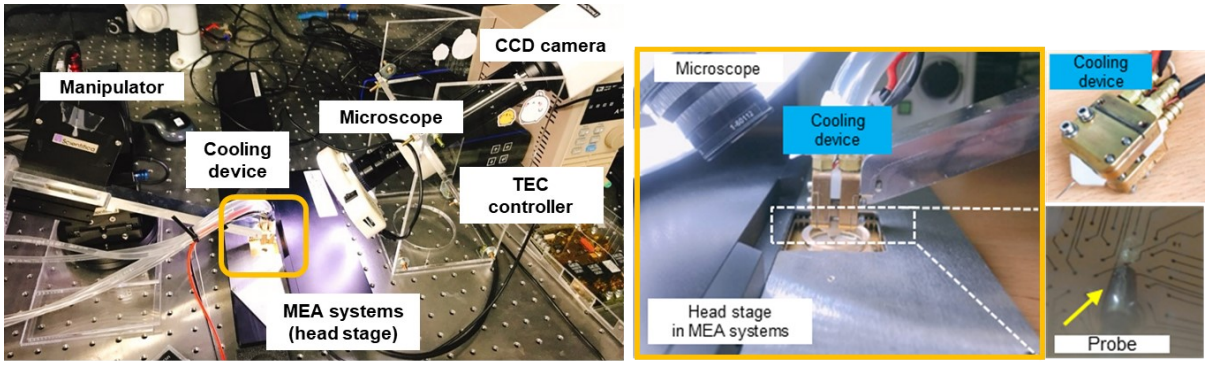
[d]*Department of Biotechnology, Yonsei University, 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea.*

[e]*Center for Nanomedicine, Institute for Basic Science (IBS), 50 Yonsei-ro, Seodaemun-gu, Seoul 03722, Republic of Korea.*

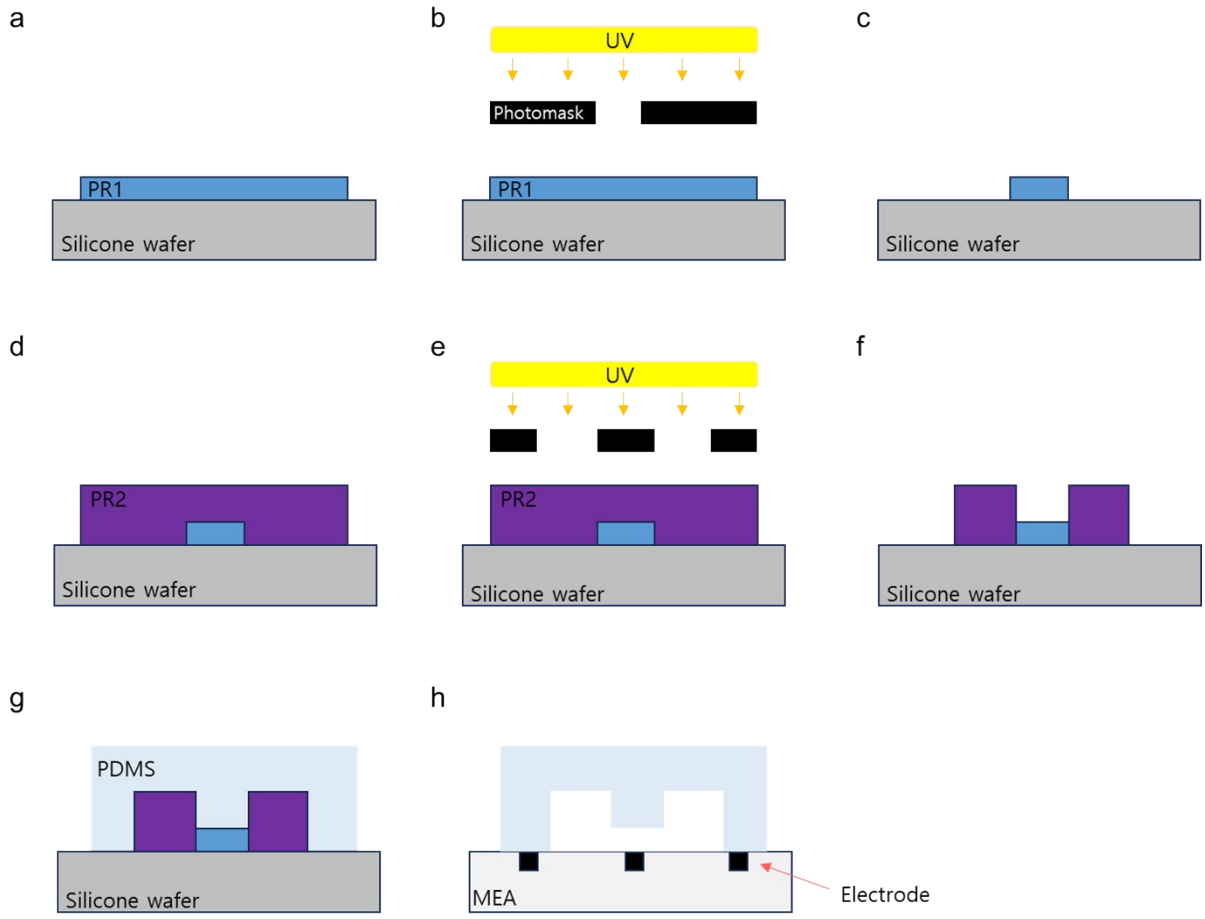
[f]*Molecular, Cellular, and Developmental Biology, University of California, Santa Barbara, Santa Barbara, CA, USA*

[g]*Research Institute for Smart Design & Manufacturing Technology, Sogang University, 35 Baekbeom-ro, Mapo-gu, Seoul 04107, Republic of Korea.*

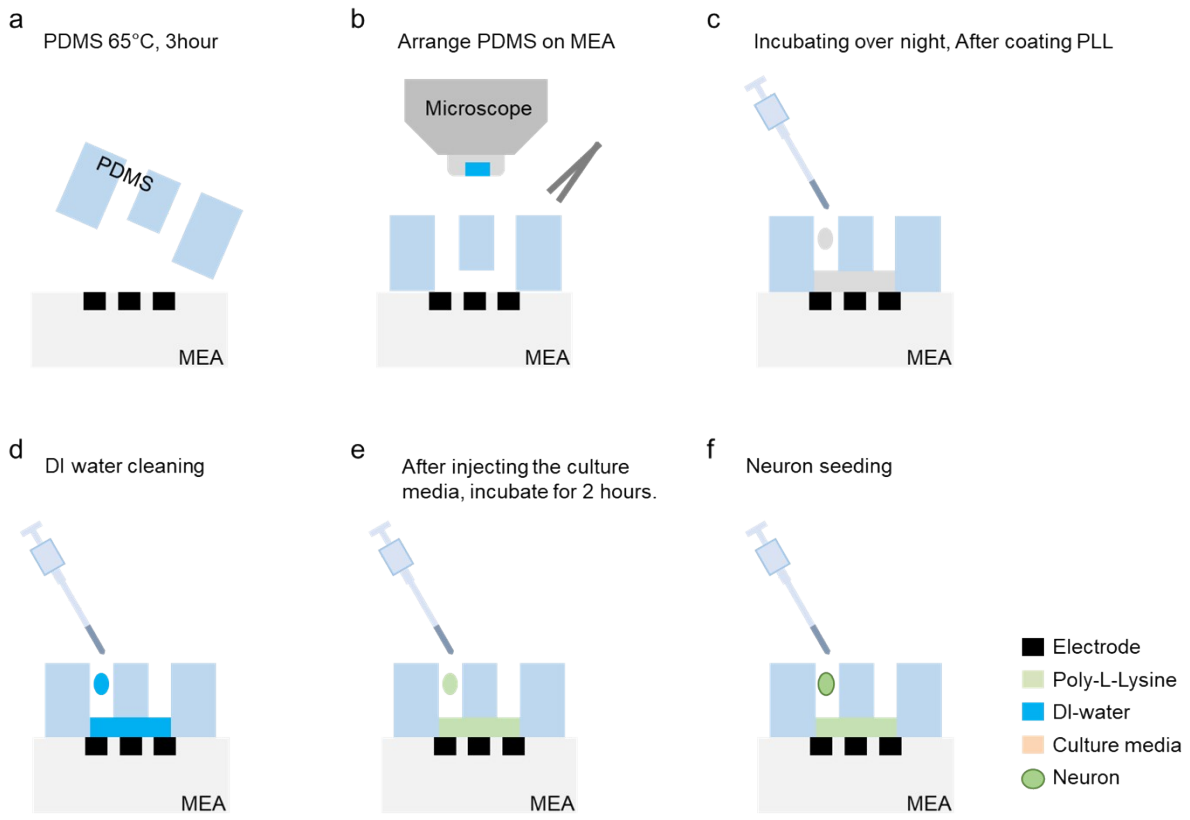
*Corresponding address: gunhokim@unist.ac.kr, sungsoo@ucsb.edu, and sortpark@sogang.ac.kr



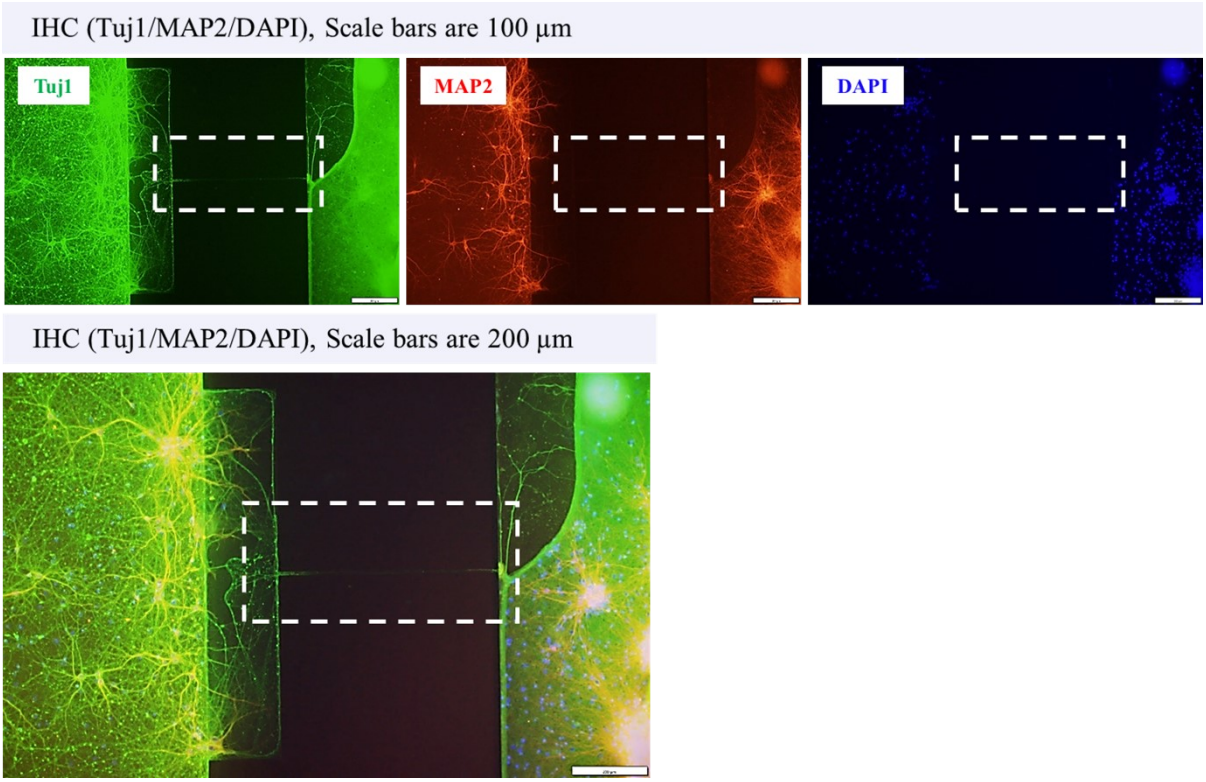
Supplementary Fig. S1. Experimental configuration.



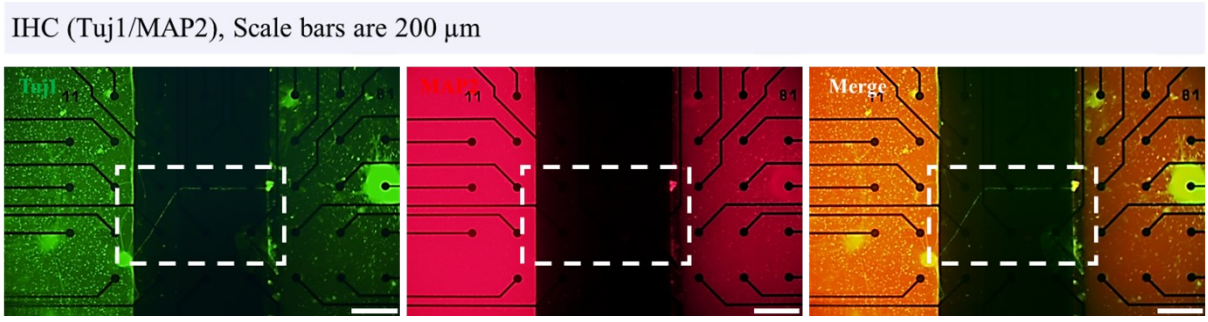
Supplementary Fig. S2. Fabrication of a cryo-neuromodulation chip (a) Deposit PR1 and spin coating (SU8 2005) (b) UV light exposure (c) Axon guide channel layer (d) Deposit PR2 and spin coating (SU8 3050) (e) UV light exposure (f) Deep channel layer (g) PDMS molding (h) PDMS on a MEA



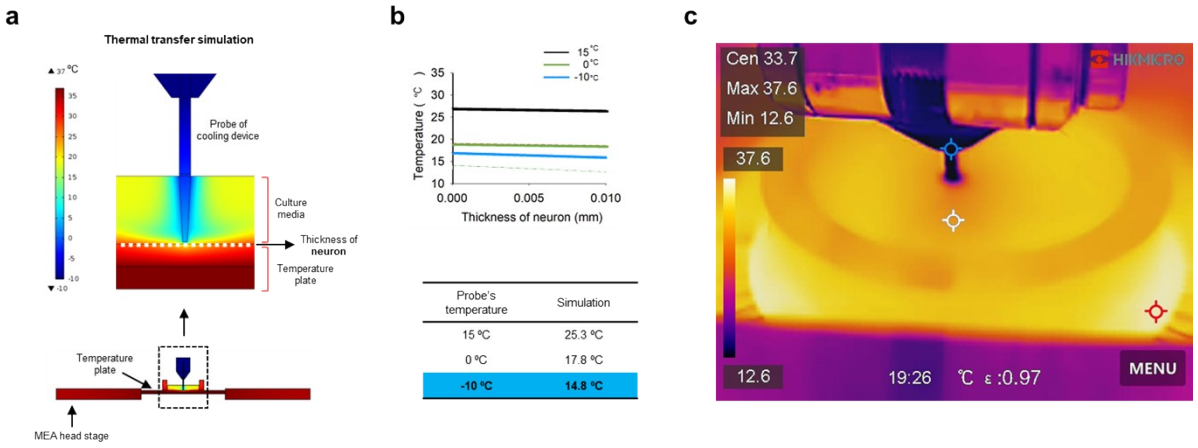
Supplementary Fig. S3. Alignment PDMS on a MEA



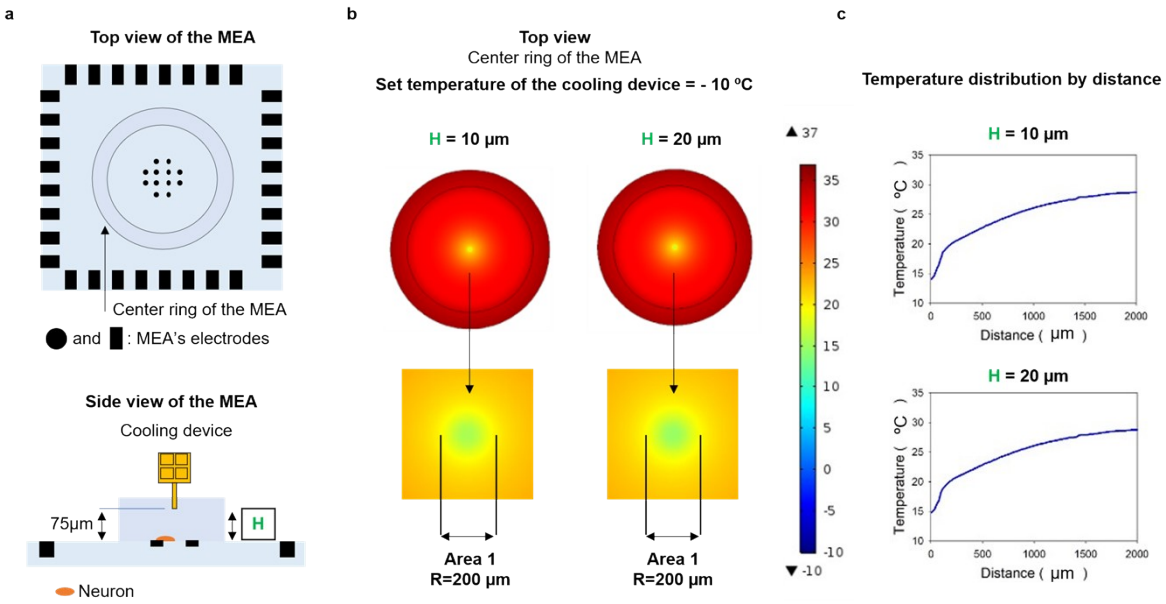
Supplementary Fig. S4. IHC staining. Neuron image on a cell culture plate (DIV 21)



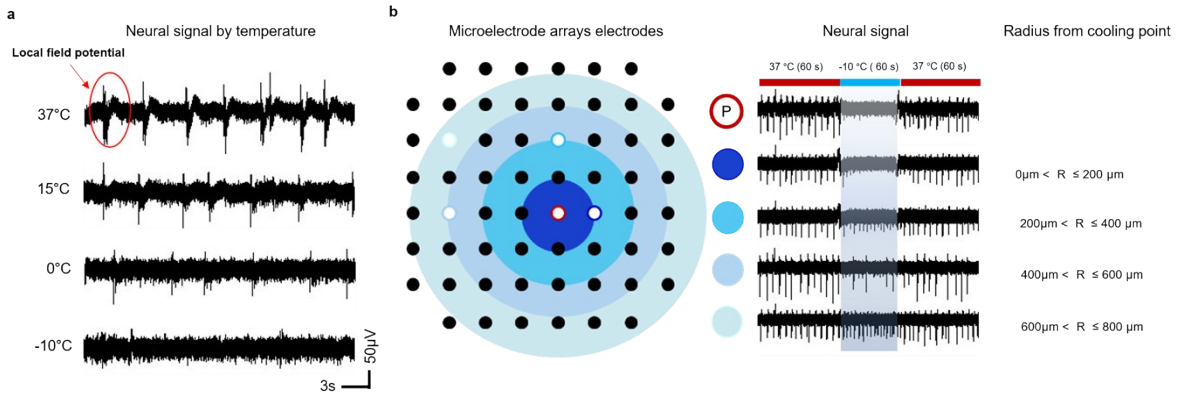
Supplementary Fig. S5. IHC staining. Neuron image on a MEA (DIV 21)



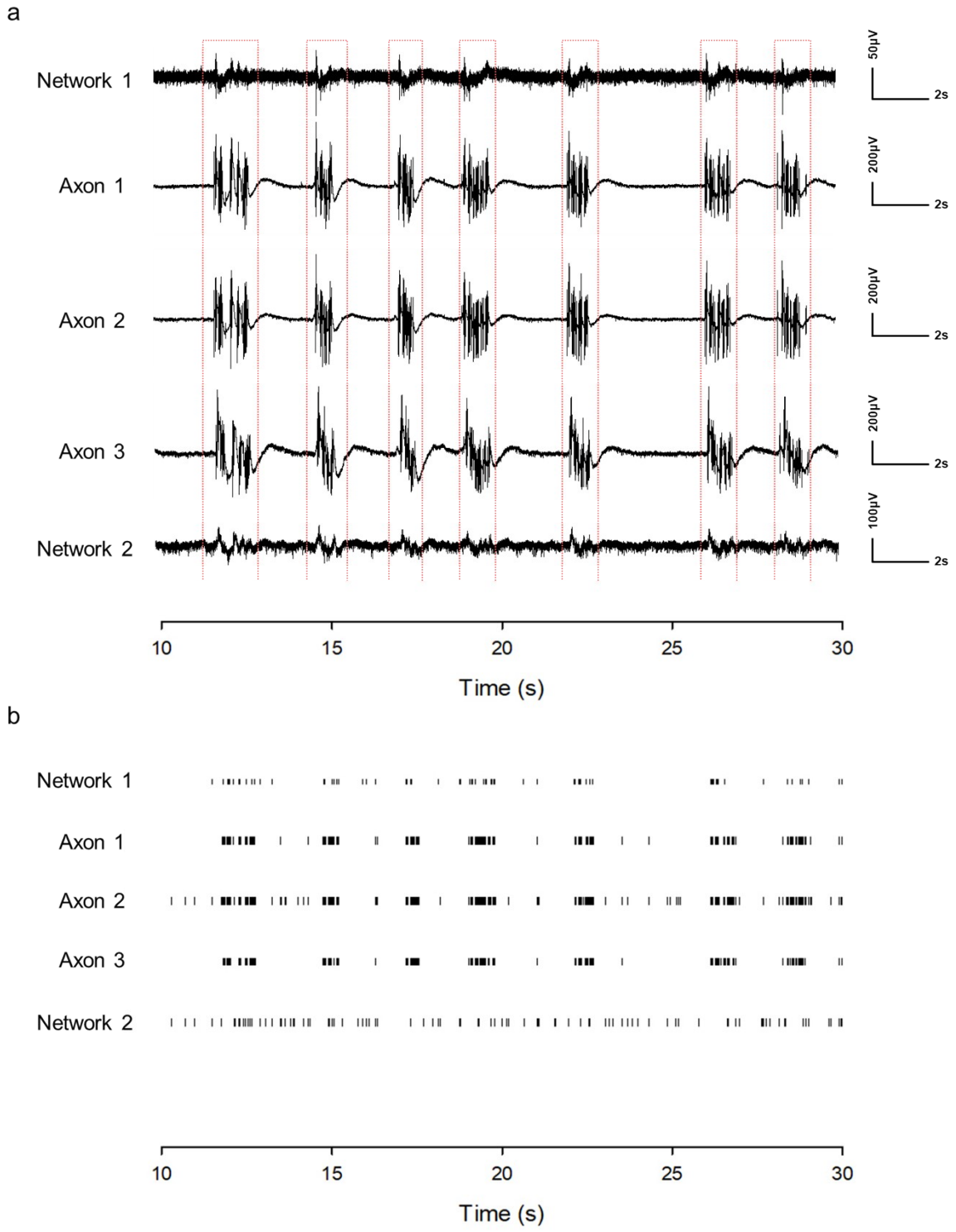
Supplementary Fig. S6. Temperature of the Cooling Device Probe: Simulation and Validation (a) Finite element simulation showing the temperature distribution around the cooling probe and neuron (b) Simulated neuronal temperature as a function of neuron thickness, along with estimated temperatures at probe settings of 15 °C, 0 °C, and −10 °C. (c) Infrared thermography image validating the localized temperature field around the cooling probe during operation.



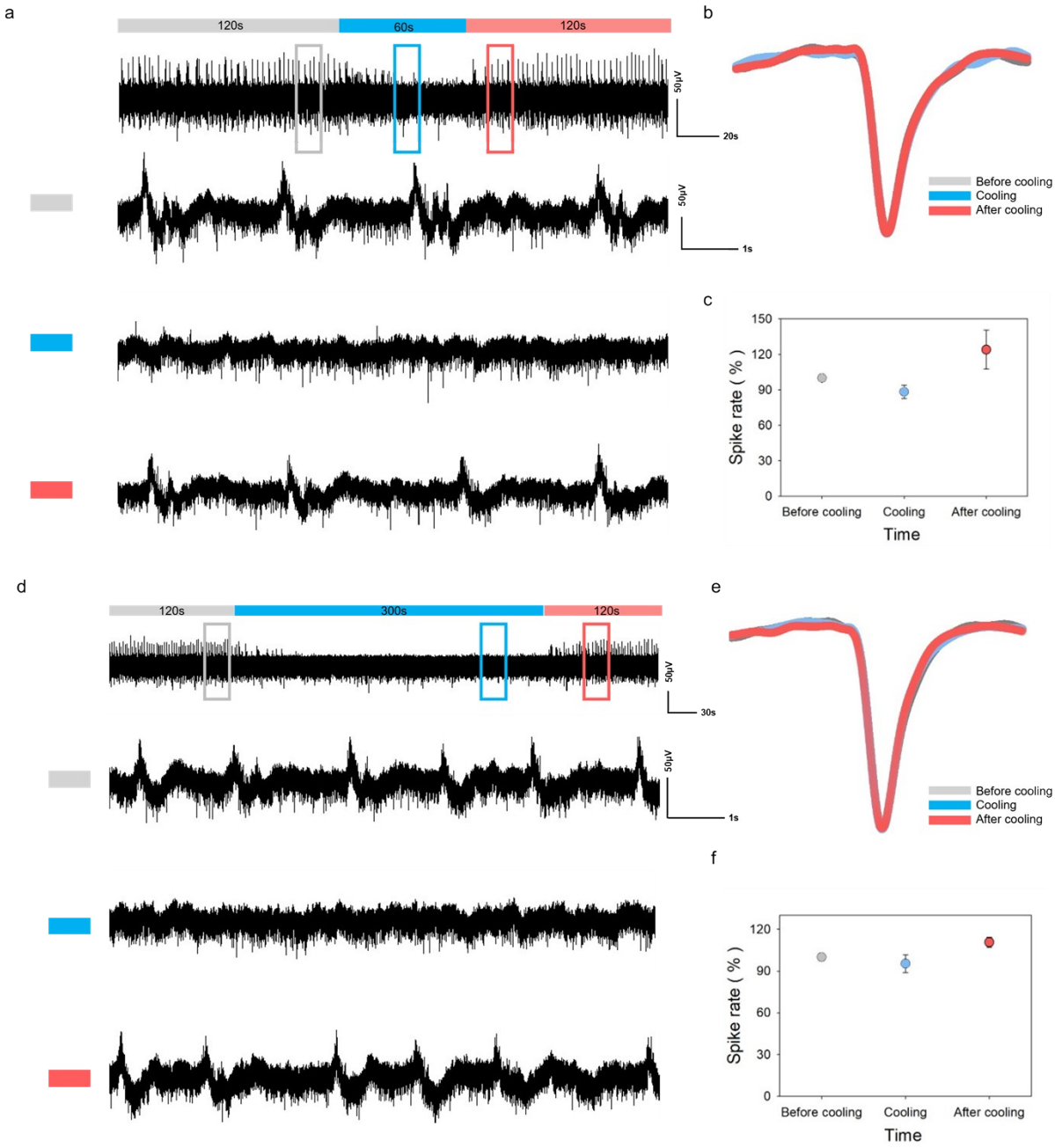
Supplementary Fig. S7. Thermal distribution simulation around the cooling probe and MEA substrate (a) Schematic illustration of the MEA (b) Finite element simulations of the temperature distribution at heights of H = 10 μm and 20 μm, corresponding to the typical soma thickness of rat cortical neurons. The cooling probe is positioned 75 μm above the MEA substrate.



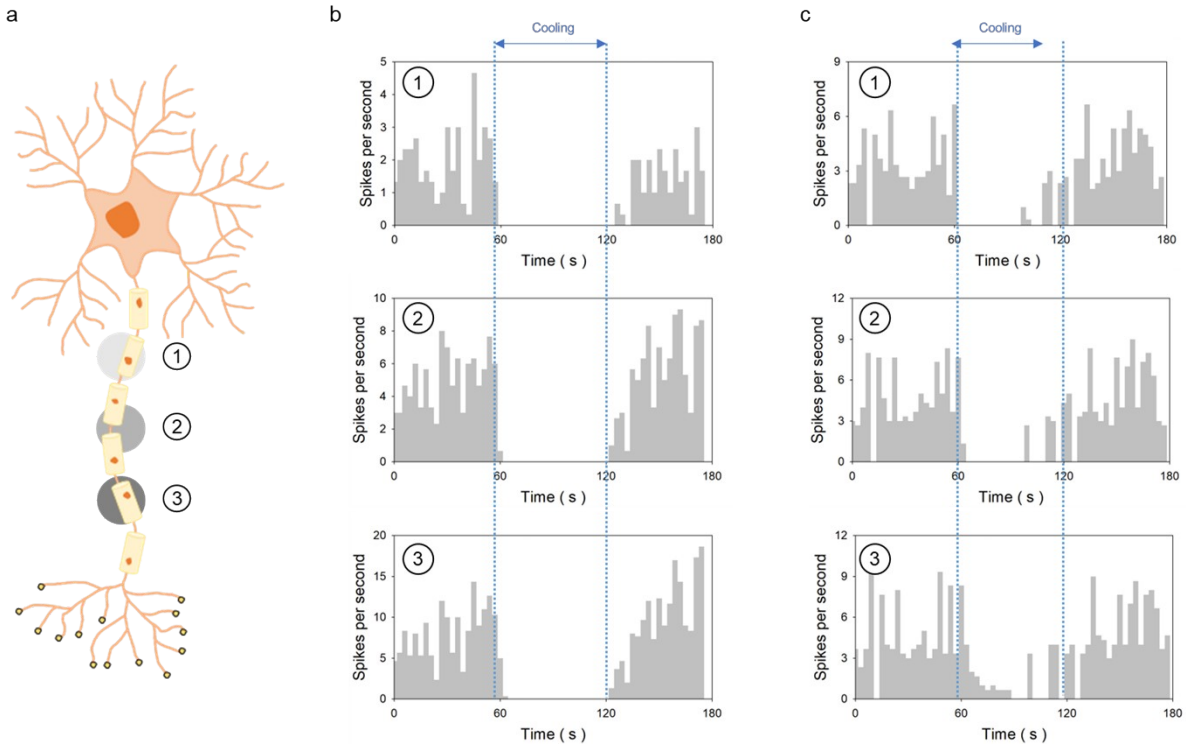
Supplementary Fig. S8. (a) Neural signals at different temperatures. (b) Neural signals by distances from the cooling area.



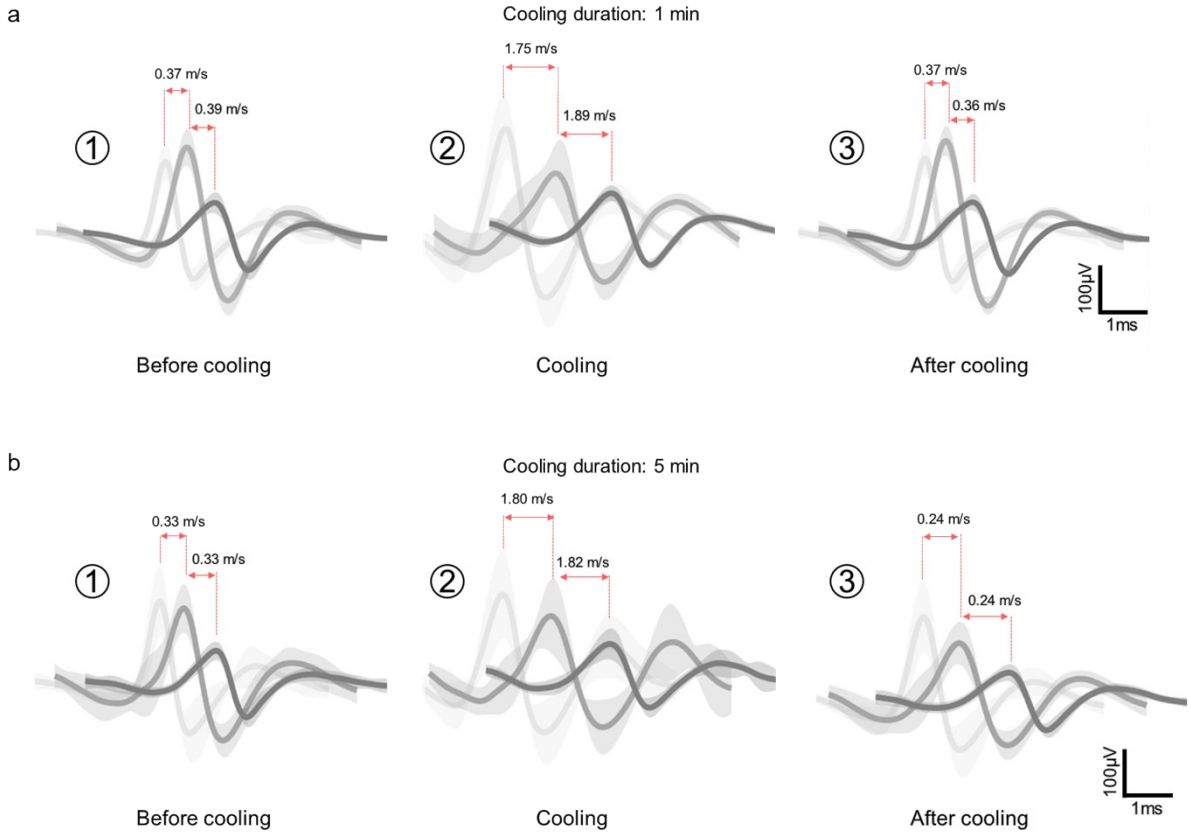
Supplementary Fig. S9. Magnified view of the yellow boxed region in Fig. 2a, highlighting synchronized burst events. (a) Enlarged view of the 10–30 s segment from Fig. 2a (The red dashed boxes indicate bursts). (b) Raster of spike time stamps.



Supplementary Fig. S10. Effects of cooling duration on spike activity in Neuron Network 2. (a) Spike raster recorded from Network 2 before, during, and after localized cooling for 1-minute. Three colored boxes (gray, blue, red) indicate representative 10-second time windows selected for spike raster analysis. (b) Average spike wave form before, during, and after cooling for 1-minture (c) Spike rate before, during, and after for 1minute (d) Spike raster recorded from Network 2 before, during, and after localized cooling for 5-minutes. Three colored boxes (gray, blue, red) indicate representative 10-second time windows selected for spike raster analysis. (e) Average spike wave form before, during, and after cooling for 5-minutes. (f) Spike rate before, during, and after for 5-minutes. Spike rate (%) = (Spike $rate_{before}$ - spike $rate_{during,\ after}$) / Spike $rate_{before}$ * 10



Supplementary Fig. S11. Axonal signal histograms (a) Schematic illustration of electrodes positioned beneath the axon. (b) Axonal signal histogram by electrode number during temperature modulation: 0–60 s at 37 °C, 60–120 s at –10 °C, and 120–180 s at 37 °C. (c) Representative results from an independent experiment under the same conditions: 0–60 s at 37 °C, 60–120 s at –10 °C, and 120–180 s at 37 °C.



Supplementary Fig. S12. Axonal conduction velocity (a) Axonal conduction velocity during 1-minute cooling at each cooling step. (b) Axonal conduction velocity during 5-minute cooling at each cooling step.