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DIRECTING SPATIOTEMPORAL FIRING PATTERNS IN PRIMARY NEURON NETWORKS USING NANOMAGNETIC FORCES

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Abstract:
Controlling the connectivity and firing patterns in neuronal networks is key to information processing in the brain. Engineering directed connectivity patterns *in vitro*, where firing patterns propagate reliably and reproducibly along a user-defined direction within a regrown neurite network, has been challenging. Recent efforts to overcome these challenges are based on using magnetic nanomaterials as a mechanical cue to orient the growth of neurite networks, *e.g.*, within magnetic field gradients. It remains, however, unclear if mechanically oriented neurite networks would exhibit a predefined connectivity or firing pattern. Here, we grew dissociated primary cortical neurons (E18, rat) on microelectrode arrays (MEA60) under two distinct mechanical force patterns (including magnetic nanoparticles). These force patterns exposed the cortical neurons to either a weak (0.3 fN/mm) or a strong force gradient (0.97 fN/mm, forces were estimated based on a single particle, 100 nm iron oxide). Directed neurite growth was assessed between two and five days *in vitro* (DIV) using neurite tracing from phase-contrast images. At eight and fourteen DIV, extracellular recordings were performed for 4 min at 10 kHz. Extracellular firing patterns were then extracted from unfiltered signal recordings and applied to Granger causality testing (Multivariate Analysis) to compute a probability of potentially directed signal causality. Probability values above $p = 0.001$ were then used to excerpt connectivity maps. Based on the connectivity maps, we found that spatiotemporal firing patterns propagate perpendicular to the gradient ascent in both the weak and the strong force gradient patterns. For our control growth conditions (no force or no magnetic field gradient), the Granger causality test indicated randomized firing patterns. These findings hold great promise to engineer neuronal growth and spatiotemporal firing patterns using nanomaterials and forces at the neuronal interface and in next-generation neurological implants.

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