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Synaptic connectivity of low density cultures of patterned neuronal networks on microelectrode arrays

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Planar microelectrode arrays permit long-term analysis of neuronal electrical activity in vitro. Our immediate goal was to determine the fewest number of neurons that can be used to form long-lived cultures to permit continuous extracellular recording and stimulation. Orthogonally patterned networks of primary rat hippocampal neurons were prepared by aligning patterns to electrodes on arrays. The crossing points were located at recording electrode sites. Poly-dimethylsiloxane stamps were fabricated to print fine poly-L-lysine patterns of 2- μ m wide lines as the connectors between 20- μ m diameter circles for neuronal processes and cell bodies, respectively. Different densities of neurons were applied on patterned arrays to establish the lowest seeding density that resulted in long-term (>14 days) functional connectivity. When cells were plated at 200 cells/mm², spontaneous activity could be recorded as early as 7 days in culture. By 21 days in culture, levels of spontaneous activity were constant. Recordings were made for as long as 71 days. Spontaneous activity could be inhibited by both glutamate and GABA receptor antagonists.

Electrical stimulation (200- μ A current step, 50- μ sec pulse width) was also used to evoke activity. The distributions of pre- and post-synaptic proteins were described using immunocytochemistry and their distribution mapped relative to cell bodies and patterns on the substrates. Results demonstrate the effectiveness and robustness of these cultures for studying network function.

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