

Organotypic co-cultures on MEA as a valuable tool to study the establishment of projection pathways in the CNS

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Injury to the adult mammalian central nervous system (CNS) caused by trauma, ischemia or neurodegenerative diseases results in serious and permanent functional deficits. Promoting repair and regrowth of neurons is an increasingly attractive approach to the treatment of neuronal injury. Evaluation of treatments that may lead to neural regeneration require model systems that closely reflect *in vivo* conditions and correlate long-term recordings of physiological activity with morphological changes. We here present an organotypic CNS culture model on micro electrode arrays (MEAs) which allows us to monitor long term effects of potential regeneration-promoting drugs on a functional level within the same culture for days to weeks.

Slices of entorhinal cortex (EC) and dentate gyrus (DG) were placed on MEAs and cultured up to 6 weeks. Connections between the two tissues were shown immunocytochemically by neurofilament staining. The functionality and specificity of the newly formed connections was shown by electrical stimulation and parallel recording of correlated activity in the target tissue. About 80% of the connections *in vitro* were unidirectional, propagating signals only from the EC into the DC. Beginning at DIV (day *in vitro*) 2, formation of functional connections took place throughout the first week *in vitro*. In addition, high frequency stimulation (100 Hz) showed characteristics of monosynaptic signal transmission, indicating that the connections between the EC and DC formed *in vitro* reflect the perforant pathway projection between the two tissues *in vivo*. Pharmacological analysis identified glutamate receptors of the AMPA/kainate type as the main mediators of excitatory signal transmission in the DG.

To evaluate our co-culture of EC and DG as a model for neuronal regeneration, we tested the impact of C3d on the formation and specificity of neuronal projections. Cd3 is a synthetic agonist of the neural cell adhesion molecule NCAM which has been shown to promote neurite outgrowth in single cell cultures. Addition of C3d to the culture medium from DIV 0 significantly accelerated the establishment of connections without affecting their specificity. The acceleration could be blocked by antagonists to PLC α and c-fyn which are key mediators of the intracellular NCAM signaling pathways.

In conclusion, we have shown that our EC-DG co-culture on MEAs is a valid *in vitro* model for the perforant pathway *in vivo* and a useful tool to investigate long term pharmacological effects on synaptic activity and regenerative processes.

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