MICROFLUIDIC CHIPS WITH "AXON DIODES" FOR DIRECTED AXONAL OUTGROWTH AND RE-CONSTRUCTION OF COMPLEX LIVE NEURAL NETWORKS

J.M. Peyrin[1], L. Saias[2], P. Gougis[2], S. Magnifico[1], S. Betuing[1], D. Kilinc[1], J.L. Viovy[2] and B. Brugg[1]

[1]CNRS, UMR7102, Université Pierre et Marie Curie, Paris, France, [2]CNRS, UMR168, Institut Curie, Paris, FRANCE

ABSTRACT

This paper reports the design of a new type of micro-fabricated device allowing the directed axon outgrowth from different neuronal subtypes and their subsequent connections to create live neural networks. Micro-fluidic cell culture systems enable the fluidic isolation of the neuronal cell bodies from their axons. Our novel chamber design enables directed axon outgrowth between different neuronal subtypes, using asymmetrical micro-channels. As a proof, we show the *in vitro* reconstruction of a directed cortico-striatal network using primary neurons.

KEYWORDS: Microfluidic, Neurons, Neuronal network, Axon outgrowth

INTRODUCTION

The brain is an exquisitely complex structure composed of many different inter-connected neuronal areas. Experimental models that are used to study brain development and/or degeneration range from whole animal models which preserve the anatomical structures but greatly limit the experimentation at the cellular level, to dissociated cell culture systems that allow detailed manipulation of cell phenotype but lack the highly ordered and instructive brain environment. This calls for the development of new experimental models that facilitate both individual cell manipulation and brain connectivity reconstruction. Microfluidics-based cell culture systems have shown to be powerful tools to handle cells in micron size environments. It was recently demonstrated that microfluidic devices composed of two fluidically isolated chambers connected by micro-channels could be used to achieve compartmentalization of neuronal cell bodies from axons [1]. Although this system allows pharmacological interventions specifically directed to axons or somata within a neuronal subtype, they do not permit efficient reconstruction of neuronal networks composed of multiple neuronal subtypes, as neurons seeded in both chambers send their axons towards the respective opposite chambers. In the mammalian brain the neostriatum is the principal recipient of afferent neurons from the frontal cerebral cortex to the basal ganglia. The main striatal targets of cortical activity are the medium spiny projection neurons that account for more than 90% of the connections. We report the design of a new microfluidic cell culture device comprising fluidically isolated chambers connected with an array of asymmetrical micro-channels. By using primary cortical neurons in one chamber and primary striatal neurons in the second, we demonstrate efficient reconstruction of directed cortico-striatal projections.

EXPERIMENTAL

Microfluidic devices were prepared by bonding polydimethylsiloxane pads created by applying standard soft lithography techniques on a glass cover slide. They consists of 1mm wide 4mm long and 55µm high culture chambers interconnected with an array of 3µm high, 500 µm long, asymmetrical micro-channels, whose width decreases from 15 µm to 3µm. After micro-dissection of embryonic mouse brain, primary cortical neurons were seeded in the first chamber and striatal neurons were seeded in the second chamber. The co-culture was maintained for up to 2 weeks, after which phenotypic maturation, synaptic connectivity and functionality was assessed using live imaging and immune-fluorescence techniques.

RESULTS AND DISCUSSION

When seeded on the chamber at the wider side of the narrowing micro-channel, mouse primary projection neurons of different subtypes (cortical, striatal, hippocampal) efficiently send axons to the opposite chamber (Figure 1); whereas when seeded in the chamber at the narrower side, they do not. This constitutes a highly selective filter, effectively operating as a diode for axonal projections. Using this directional system, we demonstrate the reconstruction of cortico-striatal projections. Cortical neurons project their axons through the narrowing channels and connect to the striatal neurons in the second chamber within 3 days in culture (Figure 1).

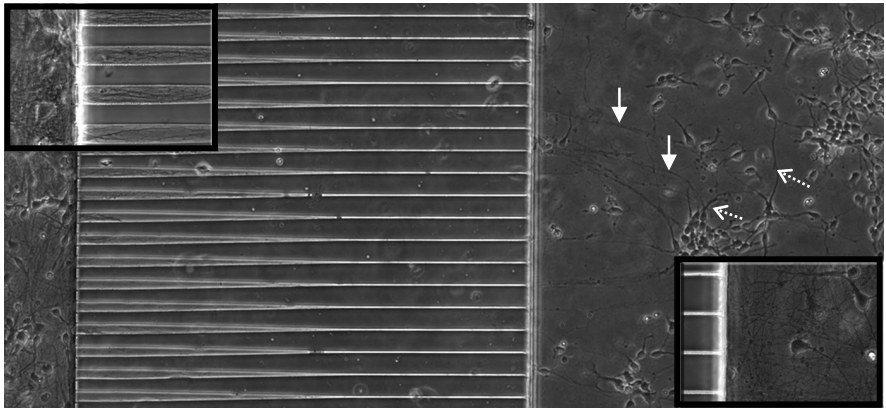

Figure 1. Mouse primary cortical neurons are seeded in the left cell culture chamber (wider side of the micro-channels). After 3 days in the culture, primary mouse neurons from the striatum are seeded in the right (distal) chamber (narrower side). 3 days after striatum seeding, cortical axons (plain arrows) connect to the striatal somas and axons (dashed arrows) (magnification X100): Left insert :Cortical neurons projecting bundles of axons in wide micro-channels. Right insert : Fascicles of cortical axons exit the narrowed channels and connect to the striatal neurons (magnification X 400).

After one week in the culture, these connections lead to the clustering of synapsin and synaptophysin proteins as an evidence for the maturation of cortical presynaptic terminals (Figure 2). Functionality of the cortico-striatal synapses was assessed using FM1-143 dyes, together with specific striatal Erk Kinase phosphorylation induced by activity dependent cortical glutamate synaptic.

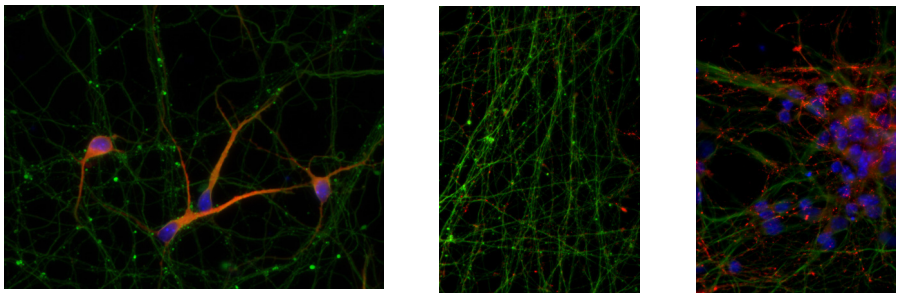

Figure 2. Left panel: Bundles of cortical axons (β3 tubulin, green) projecting to striatal neurons (MAP2, red). Central panel: Distal chamber of a mouse cortical neuron culture without any striatal neurons seeded in the chamber. Cortical axons stained for β3 tubulin (axon specific marker, green) and synapsin (synaptic marker, red). Right Panel: Distal chamber of a mouse cortical neuron culture with striatal neurons seeded in the chamber (blue nuclei). Axons of both cortical and striatal neurons are stained for β3 tubulin. Note the increase and clustering of synapsin staining (red) reminiscent of synapsin relocalization to cortical presynapic terminals. Magnification (X400).

CONCLUSIONS

Serially connecting cell culture chambers using asymmetrically narrowing, unidirectional micro-channels is a simple and powerful tool for reconstruction and manipulation of complex directional networks (such as cortico-striato-nigral projections) useful for studying developmental neuroscience as well as neurodegenerative processes, where specific subsets of neurons show early signs of synaptic dysfunction [2].

ACKNOWLEDGEMENTS

This work was supported by the French “Agence Nationale de la Recherche”.

REFERENCES

[1] A.M. Taylor, M. Blurton-Jones, S.W. Rhee, D.H. Cribbs, C.W. Cotman, N.L. Jeon. A microfluidic culture platform for CNS axonal injury, regeneration and transport. 2005, Nature Methods, 2(8), pp. 599-605.

[2] T.M. Wishart, S.H. Parson, T.H. Gillingwater. Synaptic vulnerability in neurodegenerative disease. 2006, J Neuropathol Exp Neurol. 65(8), pp. 733-9.