PERFUSION BASED MICROFLUIDIC SYSTEM FOR PHARMACOLOGICAL PROFILING OF NEURONAL NETWORKS

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ABSTRACT

This work presents the integration of a semi-automated microfluidic platform that utilizes calcium imaging to enable the pharmacological characterization of functionally connected, but environmentally isolated neuronal networks. This approach allows, for the first time, to assess the cause-effect relationship of neuronal communication following drug application, thus allowing the pharmacological characterisation of novel drugs proposed to influence communication between neuronal networks.

KEYWORDS: Perfusion, microfluidic, neuronal network, calcium imaging, glutamate

INTRODUCTION

Many *in vitro* systems fail to recreate the biological complexity of the *in vivo* neural microenvironment, which reduces the likelihood of drugs that depend on *in vitro* assays being effective during clinical trials [1]. Having previously demonstrated functional synaptic communication between independent neural networks in a microfluidic format [2], we have now integrated computer controlled fluid perfusion into the system for CNS drug discovery. The platform enables user-defined delivery of up to four different perfusates and simultaneous recordings of cellular activity via calcium imaging, from both the direct, and indirectly chemically stimulated neuronal co-cultures.

EXPERIMENTAL

Two layer microfluidic devices were fabricated using standard soft lithography procedures with PDMS devices bonded to glass coverslips, followed by UV sterilization and poly-l-lysine (10μg ml^{-1}) treatment. Mixed primary hippocampal cells were cultured in the microfluidic devices within two environmentally separated chambers that allowed synaptic connections to be formed with each other via an array of microchannels [3] (figure 1). The perfusion of multiple compounds in one chamber (direct stimulation) was achieved using computer controlled syringe driven perfusion pumps which were connected to the inlets/outlets of the microfluidic device on the day of experimentation. Live/dead assays were performed on microfluidic cultures following perfusion across a range of flow rates with cell death determined using live/dead staining with hoechst and propidium iodide. Characterisation of perfusion in the microfluidic device was performed using calcein (50μM), and the pharmacological profiling of glutamate activity achieved using a range of glutamate concentrations and a combination of ionotropic antagonists.

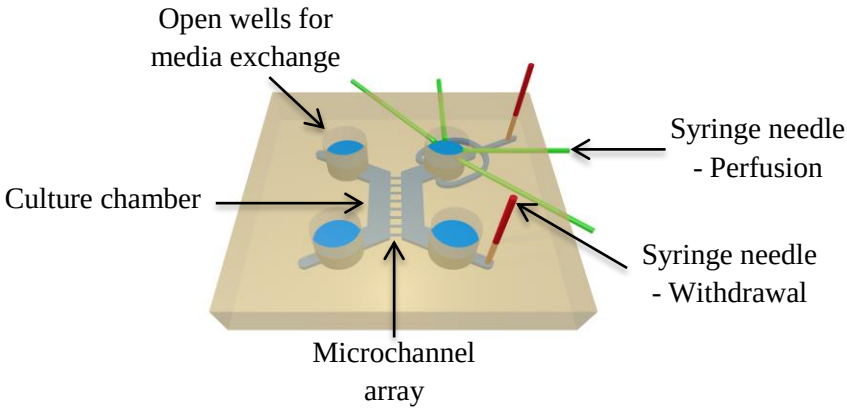

Figure 1: Dual chamber microfluidic device, modified with inlets and outlets for the semi-automated perfusion and withdrawal of fluid

RESULTS AND DISCUSSION

Perfusion of calcein across multiple recordings revealed consistent wash on/off fluorescent profiles in the absence of fluid cross contamination between perfused and non-perfused chambers. This indicates that any changes to indirect neuronal activity is a consequence of functional synaptic communication between the two neuronal networks. A flow rate of 4μl min^{-1} preserved cell health in both exposed and naïve neural networks following live/dead assays, and a glutamate concentration response curve following direct stimulation revealed an EC_{50} of 4μM. Pharmacological manipulation of neuronal activity was also achieved as the neuronal response to glutamate (3μM) was reversibly reduced in the presence of glutamate antagonists (NBQX/DL-AP5/MCPG 20/100/500μM; Figure 2). This demonstrates, as a proof of principle, that this system is capable of carrying out pharmacological profiling of novel drugs.

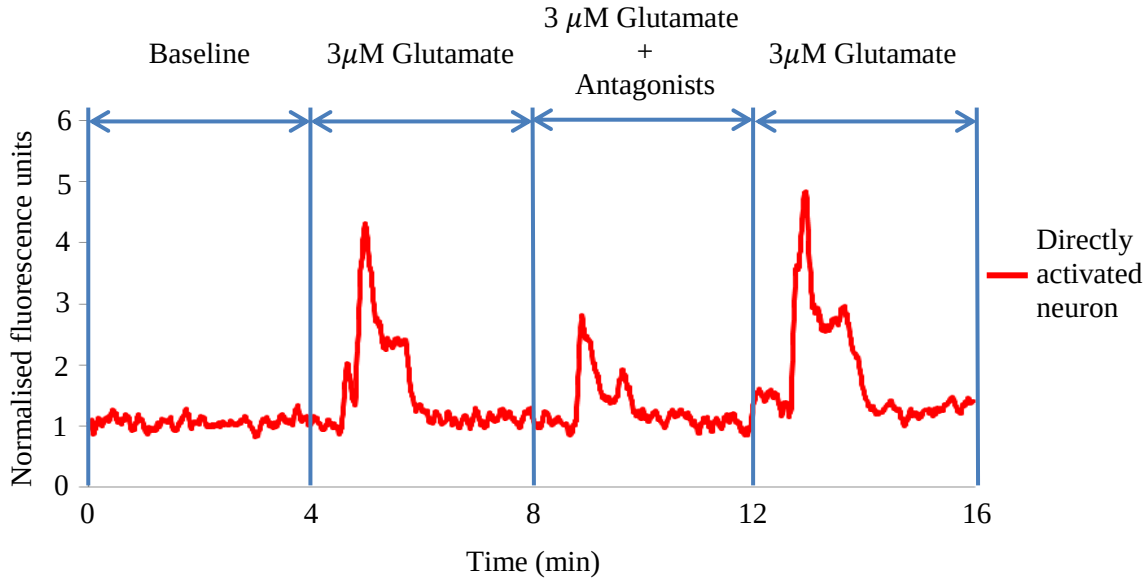

Figure 2: Reversible inhibition of neuronal activity in response to glutamate perfusion

CONCLUSION

Mapping the direct/indirect neuronal network response to drug application will allow a better understanding of the pharmacological profile of new drugs and how they influence the communication between neuronal networks. The novel microfluidic platform presented here produces high-throughput pharmacological data for agonist/antagonist compounds in a neurological setting and has the potential to be used for investigating the therapeutic potential of novel compounds for CNS disorders.

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