Dual-compartment microfluidic device for neuronal co-cultures: Design, implementation and validation
Kanagasabapathi, T.T.

Citation for published version (APA):
Kanagasabapathi, T. T. (2012). Dual-compartment microfluidic device for neuronal co-cultures: Design, implementation and validation

General rights
It is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), other than for strictly personal, individual use, unless the work is under an open content license (like Creative Commons).

Disclaimer/Complaints regulations
If you believe that digital publication of certain material infringes any of your rights or (privacy) interests, please let the Library know, stating your reasons. In case of a legitimate complaint, the Library will make the material inaccessible and/or remove it from the website. Please Ask the Library: http://uba.uva.nl/en/contact, or a letter to: Library of the University of Amsterdam, Secretariat, Singel 425, 1012 WP Amsterdam, The Netherlands. You will be contacted as soon as possible.
Chapter 1

Introduction
1.1 Preface

Understanding electrophysiological processes at network level is one of the major challenges in current neuroscience. Despite the fact that in vivo brain studies preserve structural relationship between different regions of the brain and provide information on the functionality of brain network, chemically manipulating a specific region of the brain in vivo introduces a high degree of complexity.

The massive connectivity and limited spatial resolution for simultaneously recording from multiple regions of the brain, restricts the possibility to study the interaction between specific regions of the brain or to study the influence of a particular region on the other without influence from external connections in vivo. Although organotypic slices provide better spatial resolution for electrophysiological recordings, selective manipulation (electrical and chemical) of a specific region in an organotypic slice is often not possible. Hence, developing a co-culture system with dissociated cells in compartmented devices may provide better manipulation capabilities that compliments in vivo and organotypic slice studies. This may provide an interesting intermediate level for understanding electrophysiological processes and signal propagation at network level between two or more different sub-populations of neurons.

Small ensembles of neuronal populations can be integrated by using dissociated neuronal cultures coupled to microelectrode arrays (MEA). The functional characteristics of the MEA permit mid to long-term recordings (weeks to months) of both spontaneous and evoked neuronal network activity patterns and of their spatio-temporal evolution (Potter and DeMarse 2001; Morin, Takamura et al. 2005; van Pelt, Vajda et al. 2005). This allows investigating network development (Van Pelt, Corner et al. 2004), network level effects of different neuroactive compounds and pharmacological substances (Gramowski, Jugelt et al. 2006; Morin, Nishimura et al. 2006) and regulation of neuronal excitability (Shu, Hasenstaub et al. 2003; van Pelt, Vajda et al. 2005). When combined with techniques to isolate neuronal sub-population, cell cultures provide an excellent environment for controlling aspects of the experiment that one could not control in vivo.

This thesis describes the design, implementation and validation of a dual-compartment device for co-culturing dissociated neurons that can be functionally connected across a diffusion barrier and can be pharmacologically manipulated at the level of each individual sub-population. An experimental approach to selectively manipulate individual cell type and/or connections using neuro-active pharmacological substances and their influence on signal propagation between the regions of the co-culture is presented. Cortical and thalamic dissociated cells were used to develop a
sample co-culture system that can be individually manipulated.

In vivo studies have recognized thalamus as a site for two-way interaction with the cortex more than as a mere relay station of information (Miller 1996). Thalamic neurons receive substantially strong input from cortico-thalamic feedback neurons thereby allowing the context to communicate continuously through the thalamus during sensory processing (Miller 1996). The influence of cortical cues in the development of thalamo-cortical connectivity or in the remodeling of networks following environmental modifications is of interest (Coronas, Durand et al. 2000). Network interaction between cortex and thalamus is important in understanding many fundamental brain functions such as sleep modulation, sensor-motor information integration and the transition of spike-wave seizures in epilepsy model.

Although, the in vitro system demonstrated in this work does not mimic the complex three-dimensional in vivo conditions, microfluidic separated dual compartments coupled to MEA might open new perspectives in the study of network-level interaction between sub-populations. For instance, in a cortical-thalamic circuitry, the dynamics in increased cortical excitability leading to the transition of spike-wave seizures by selective application of neuroactive GABA antagonist to cortex region alone can be studied using the co-culture system reported in this work.

1.2 Compartmented System for Dissociated Neuron Cell Culture

Compartmentalization of cells and controlling the network connectivity may provide an ideal tool for developing in-vitro neuronal pathways. Maher et al demonstrated a well structure that holds the cell in close proximity to a metal extracellular electrode while permitting normal outgrowth of axons and dendrites (Maher, Dvorak-Carbone et al. 1999) and further demonstrated a technique to capture neurons in the immediate vicinity of the electrode by providing physical isolation (Maher, Pine et al. 1999). Similarly, other techniques such as dielectrophoresis, chemical surface patterning, PDMS stencils and micro-contact printing were used to provide successful cell isolation (Heida, Rutten et al. 2001; James, Spence et al. 2004; Morin, Nishimura et al. 2006; Sorkin, Gabay et al. 2006; Jun, Hynd et al. 2007; Nam, Brewer et al. 2007).

In this thesis, I designed and implemented a two compartment system on a MEA substrate that supports small assemblies of different interconnected neuronal cell types.

The emphasis of the research is to demonstrate co-culturing physically separated yet functionally connected sub-populations of cell types in a dual compartment microfluidic device. The main advantage of such a microfluidic system lies in its
ability to pharmacologically manipulate the individual compartment of the co-culture selectively.

We chose cortical and thalamic cells for co-culture studies in our experimental approach because they form a neuronal system with a unique interaction in its sub-circuitry. Conclusions from the present work may be used to investigate the interplay in network dynamics and connectivity in the cortical–thalamic system. This in vitro dissociated cell culture system offers a complimentary approach to both in vivo and brain slice studies.

The questions I set out to address are:

1. Can we develop an in vitro dissociated cell culture model that facilitates studying a sub-circuitry or sub-population of cells from different brain regions with capabilities to selectively manipulate individual region?

2. What are the device requirements to develop such a system? Will the device be able to provide somatic and fluidic separation between different cell types while providing means for synaptic connectivity between them? How well can we influence the viability of cells in such a system?

3. How to characterize the functional connectivity between the regions and what do we learn from them? (i.e.: How useful is network cross-correlation analysis to quantify functional connectivity?)

4. Does the functional connectivity within a region differ from those across the region? How can we characterize the directionality in signal propagation between the regions?

5. How can we characterize the influence of a specific cell type in the sub-circuitry of the cortical-thalamic co-cultures? What is the directionality in signal propagation between cortical and thalamic cell types?

6. Selective pharmacological manipulation of the co-culture may provide evidence to validate the influence of particular cell type and the directionality in signal propagation between sub-populations. Can the system provide capabilities to manipulate individual regions pharmacologically?
1.3 **Microfluidics Meets MEA Technology**

Multielectrode arrays (MEA) provide a means for simultaneous extracellular recording and stimulation of several individual neurons in culture (Pine 1980; Brekeenridge, Wilson et al. 1995; Morin, Takamura et al. 2005). MEA enable long-term monitoring of the electrophysiological activity of both dissociated cells and slices, and provide a unique window to observe spatio-temporal patterns of activity in intact two-dimensional layers of neurons. MEA are used to address the need for an intermediate level of investigation between single cell patch-clamp studies and complex in vivo studies, whereby the effects of tampering with ion channel function could be measured on small functional cellular structures and networks of affordable complexity.

Considerable progress has been made in the development of traditional MEA system over the past decade and they are making inroads into pharmaceutical drug discovery and neurotoxicity testing (Gopal, Miller et al. 2007; Radio and Mundy 2008). MEA system provides means for non-invasive characterization of neuronal response. When combined with state of the art microfluidic devices, the MEA systems offer far more capabilities by providing a platform for segregating cells into separate regions, providing controlled nutrient supply to cell types and controlled pharmacological manipulation in combination with electrophysiological recording and stimulation, enabling new kinds of experimental studies not possible with the current two dimensional microelectrode arrays.

Microfabrication technologies (**Box 1**: Microfabrication process steps), from the semiconductor processing industries, has largely been adopted in the field of microfluidics for realizing devices with miniaturized channels and compartments in the order of few microns to millimeters. Microfluidic devices require low volume samples (i.e. several parallel experiments can be performed with very small quantities of brain tissues and hence, fewer animals are sacrificed), properties of fluid at microscale could provide stable and uniform micro-environment for the cells, medium throughput, measurement efficacy and better sample handling compared to the macroworld.

The volume of sample required are in the order of microlitres and because of the efficiency in sample handling, microfluidic devices are increasingly used in applications requiring biological samples. For our specific application, microfluidics offer fluidically isolated subcellular compartments that are interconnected through microchannels. With the advent of soft/rapid lithography technique as reported by Whitesides et al., (Duffy, McDonald et al. 1998; McDonald, Duffy et al. 2000; McDonald and Whitesides 2002) polymers such as polydimethylsiloxane (PDMS),
Box 1: Microfabrication process steps for making dual-compartment device

(A) Fabrication of 3 µm thick SU-8 photoresist layer for microchannels;
(B) Fabrication of second SU-8 layer of 100 µm thick for compartments;
(C) Prepolymer of PDMS was poured on the Si wafer containing negative replica of the compartments;
(D) Solidified PDMS layer was peeled-off from the Si wafer;
(E) Reservoir holes were made in the PDMS using laser machining;
(F) The polymerized PDMS layer was reversibly bonded to MEA substrate after hydrophilization.
poly(methylmethacrylate) (PMMA), polyurethanes and polymides are favored as materials of choice for creating microfluidic structures. PDMS elastomer (commercially available as Sylgard 186 from Dow Corning, US) is in particular most widely used due to its physical and chemical properties, biocompatibility, ease of fabrication and flexible surface chemistry (McDonald and Whitesides 2002; Ng, Gitlin et al. 2002; Sia and Whitesides 2003; De Silva, Desai et al. 2004; Matsubara, Murakami et al. 2004; Peterson, McDonald et al. 2005; Gross, Kartalov et al. 2007). PDMS in its cured form is mainly an oligomer terminated with a vinyl group. When PDMS elastomer is prepared by using the prepolymer and the curing agent, not all the oligomer strands are incorporated into the cross-linked network upon curing (Lee, Park et al. 2003). Uncross-linked, low molecular weight oligomers are present in the bulk PDMS. High thermal stability, chemical resistance, low electrical conductivity and hydrophobic surface characteristics of PDMS are attributed to this unique chemical structure. Further, exposing PDMS surface to oxygen plasma destructs methyl group (Si-CH3) and introduces silanol (Si-OH) group on the surface which renders it hydrophilic (Box 2: Oxygen plasma treatment of PDMS compartment). This flexible surface chemistry allows PDMS to be used in wide variety of biological assays requiring laminar flow through the microchannels.

Taylor et al. (Taylor, Rhee et al. 2003) pioneered a PDMS based dual compartment device that allowed compartmentalization of neuronal cells and control of fluidic microenvironment. The unique design of this device with two compartments of height 100 µm each separated by microchannels of 10 µm wide and 3 µm heigh provided the necessary fluidic and neuronal soma separation while providing necessary access path for neurites to cross-over. However, co-culturing sub-population of cells (i.e. neurons and glia) in the dual compartment device has been demonstrated only recently (Majumdar, Gao et al. 2011).

Integration of PDMS compartmented devices with MEA and electrophysiological recording of neuronal spontaneous activity from the sub-population in device has not been demonstrated earlier. Further, co-culturing sub-populations of cells in the dual compartment device integrated with MEA, establishing functional connectivity between them and selectively manipulating an individual cell type has also not been demonstrated elsewhere. In the experimental approach presented in this thesis, we demonstrated a MEA integrated dual-compartment device for co-culturing and electrophysiological characterization of two different cell types (i.e. Cortical and Thalamic). Furthermore, we also demonstrated capabilities to manipulate individual regions selectively.
Box 2: Oxygen plasma treatment of PDMS devices

With the oxidation process silanol groups (Si-OH) are formed on the non-patterned surface making it hydrophilic (see Figure).

![Figure 1.3. Oxygen plasma treatment of PDMS surface.](image)

Temperature cured PDMS devices were temporarily transferred to microscope glass slides to avoid hydrophilization of contact surface. Hydrophilizing contact surface results in medium leakage through the under surface of PDMS and hence, only the inner surface of the compartments and the microchannels are hydrophilized. Access path for plasma to the inside of the chamber is provided by the four open reservoirs (Figure 1.4). Once the surface is hydrophilized, PDMS devices were transferred on to PEI coated MEA substrates. The hydrophilization process enables laminar flow and easy filling of cell suspension in the compartment.

![Figure 1.4. Oxygen Plasma treatment on PDMS. The plasma reaches the surfaces to hydrophilize the four reservoirs and the inner surface of the compartments.](image)
1.4 **NEUROFLUIDIC COMPARTMENTED DEVICE TECHNOLOGY**

When combined with substrate integrated planar MEA, compartmented devices provide a suitable platform for measuring electrophysiological activity of compartmentalized neuron and studying the interaction between cells in compartment. Use of interconnected micro-chambers that allow compartmentalization of cells and control of the fluidic environments would present several possibilities for coculture studies and selective pharmacological manipulation. First attempts to develop neuron cell cultures in isolated compartments were reported in the pioneering work by Robert Campenot (Campenot 1982). Campenot chambers permit the isolation of cell bodies and to control the local fluid environment of somata. Starting from Campenot’s pioneering research, compartmentalization has been widely used to study the formation and maintenance of neuronal projections, suitable to create isolated fluidic environment and to selectively expose neuronal cells to biochemical cues and neurotransmitters (Bussiere, Vance et al. 2001). In Neurochips (Maher, Pine et al. 1999), small silicon micromachined cages are formed around individual electrodes in which a single cell body can be placed. The device was used for one-to-one correspondence of neurons to electrodes and to establish bidirectional electrical contacts with individual cultured neurons. Using dielectrophoretic fields to selectively force neutral, polarizable biological cells to fields of maximum and minimum field intensities, Heida et al., (Heida, Rutten et al. 2001; Heida, Vulto et al. 2001; Rutten, Mouveroux et al. 2001) demonstrated techniques to trap neural cells in two separate regions. On the other hand, chemical surface patterning (Ravula, McClain et al. 2006) has been performed by a variety of techniques (Ruardij, Goedbloed et al. 2000; Rutten, Mouveroux et al. 2001; James, Spence et al. 2004; Morin, Nishimura et al. 2006; Jun, Hynd et al. 2007; Rajaraman, Choi et al. 2007) to successfully pattern Poly-Lysine, Laminin, Collagen, and polyethylenimine on top of microelectrode arrays to segregate cells at network level. Adhesion and patterning of cortical neurons was investigated on isolated islands of neuron-adhesive polyethylenimine (PEI) surrounded by a neuron-repellent fluorocarbon (FC) layer and the development of fasciculated neurites between the PEI-coated areas was studied (Ruardij, Goedbloed et al. 2000). Although these techniques make it possible to reconfigure the connectivity of the networks within a culture, fluidic isolation between individual regions and selective pharmacological manipulation of the individual region was not successful with these approaches.

Integration of microfluidic structures on planar MEAs for the physical isolation of neuronal cells has recently been demonstrated (Claverol-Tinture and Pine 2002; Bani-Yaghoub, Tremblay et al. 2005; Morin, Takamura et al. 2005; Berdondini,
1.6 Use of Co-Culture Studies

Chippalone et al. 2006; Morin, Nishimura et al. 2006; Ravula, McClain et al. 2006; Dworak and Wheeler 2009; Majumdar, Gao et al. 2011; Pan, Alagapan et al. 2011). However, co-culturing subpopulation of dissociated cells for long term studies (up to few weeks), providing complete fluidic isolation between compartments, recording electrophysiological activities simultaneously to establish functional connectivity between the sub-populations and selectively manipulating an individual region has not been reported earlier. In this thesis, a technique to integrate closed interconnected micro-chambers on planar MEAs that allow compartmentalization of neuronal cells and control of the fluidic environment is presented.

1.5 Dissociated Cell Culture

Dissociated neuronal cultures have been used in many network electrophysiological studies mainly owing to the excellent accessibility offered by these systems compared to the in vivo approach. Dissociated cultures are used for investigating the cell biology of neurons and synapses, reducing the complex 3-Dimensional brain tissue to providing a relatively homogeneous population of neurons in two dimension, and facilitating physical and chemical access to neurons (Ramakers, Raadsheer et al. 1991; Muller, Swandulla et al. 1997; Bi and Poo 1998; Misgeld, Zeilhofer et al. 1998; Jimbo and Robinson 2000; Latham, Richmond et al. 2000). Although, much of the native connectivity is lost in the process of preparing the dissociated culture, studies involving electrophysiological recording and stimulation of dissociated cells, pharmacological manipulation and imaging have resulted in describing fundamental properties of network response (Maeda, Robinson et al. 1995; Beggs and Plenz 2004; Van Pelt, Corner et al. 2004; Wagenaar, Pine et al. 2004; Wagenaar, Nadasdy et al. 2006), learning in vitro (DeMarse, Wagenaar et al. 2001; Shahaf and Marom 2001; Marom and Shahaf 2002; Eytan, Brenner et al. 2003; Bakkum, Shkolnik et al. 2004; Marom and Eytan 2005; Ruaro, Bonifazi et al. 2005; Li, Zhou et al. 2007) and pharmacological testing (Pancrazio, Whelan et al. 1999; Morefield, Keefer et al. 2000; Chiappalone, Vato et al. 2003). In vitro approaches offer high degree of accessibility to pharmacological manipulations compared to intact animals and they can be used for drug discovery and neuro-toxicological studies. Although the dynamics of the nervous system under in vitro circumstances in a 2D substrate are different from those in live animals, studies have demonstrated that dissociated cultures retain their specific phenotype, receptor properties, synaptic mechanism, response to neuroactive compounds and inherent spontaneous electrophysiological activity (Dichter 1978; Brodie, Bak et al. 1986; Ichikawa, Muramoto et al. 1993; DeLima, Merten et al. 1997; Chuckowree and Vickers 2003; Haas, Vickers et al. 2004).
Though the complexity and synaptic connectivity dynamics in vivo may not be fully replicated, in vitro synaptic connectivity is functional and may undergo various forms of synaptic plasticity. A system that provides tools for monitoring and manipulating basic mechanism of cells and networks in culture may provide good understanding of network level signal propagation. To this effect, dissociated cell culture systems provide means to precisely manipulate neurons, parts of a network or its synaptic connections and thereby facilitate insights into the functioning of the system.

When cells from the embryonic brain are dissociated and cultured, neurons that have completed division will extend processes and become excitable. A dissociated cell culture is prepared from suspensions of individual cells from neural tissue. When plated onto a substrate, the neurons begin to extend processes within several hours and form a dense network. During the first few days in culture individual neurons can be seen and the preparation allows direct observation of growing neurites as they continue to branch. Under certain conditions, it is possible to maintain these cultures for long term studies (for up to few months) (Potter and DeMarse 2001). Cultured neurons are spontaneously active, and show complex patterns of electrophysiological spike and burst activities (Martinoia, Massobrio et al. 2004; van Pelt, Vajda et al. 2005). Spontaneous neuronal activity generally appears from 5 to 7 days-in vitro and the networks remain active and pharmacologically responsive for an extended period of time (Potter and DeMarse 2001).

**Electrophysiological Measurements From Dissociated Cells**

It is possible to extracellularly record the electrical fields generated by action potentials in the neurons in culture (Box 3: Extracellular recording of action potential). The level of activity of dissociated cortical cells in culture is characterized by stochastic spontaneous spiking in the early stages of development in vitro (first week) which changes to organized bursting in the later stages (second week).

Maturation of the network is characterized by the correlation in network wide non-periodic, synchronized bursting activity of various size, shape and interval distributions with minute-to-minute fluctuations in the probability of firing (Habets, Vandongen et al. 1987; Kamioka, Maeda et al. 1996). Synchronized network bursting is recognized to be a peculiar feature of systems that have neither exterior input nor output as in the dissociated cell cultures (Corner, van Pelt et al. 2002; Wagenaar, Madhavan et al. 2005).
Extracellular recording of action potential with planar microelectrode\textsuperscript{1}. When the cell body is partially covering the electrode surface and the remaining free electrode area is in contact with the culture medium and an external reference electrode. The amplifier connected to the microelectrode records the sum of the potentials at the surface of the free electrode area and the surface of the electrode covered by the cell membrane. The voltage recorded by the amplifier between the microelectrode and the reference electrode can be calculated.

1. Advances in network electrophysiology, Makoto Taketani, Michel Baudry, Springer 2006
CHAPTER 1

Introduction

In vitro bursts recorded from one electrode consist of fast sequences of multi-unit spikes with a short inter-spike interval (ISI) separated by an interval that is relatively long compared to the burst duration (van Pelt, Wolters et al. 2004; Chiappalone, Novellino et al. 2005). When the bursting behavior is organized in network-wide regions involving entire or part of the network at the same time, it is described as network burst (van Pelt, Wolters et al. 2004). Network bursts consist of sequences of synchronized single-channel bursts, which spread across all or part of the MEA (van Pelt, Wolters et al. 2004; Eytan and Marom 2006; Raichman and Ben-Jacob 2008). Extracellular action potentials and network-wide burst response from cell cultures were similar to those recorded in vivo (Maeda, Robinson et al. 1995; Wagenaar, Pine et al. 2006). Spike trains recorded from electrodes in both compartments were used to compute correlation in network behavior. Utilizing cross-correlation based methods (Garofalo, Nicus et al. 2009), we analyzed correlation in spontaneous network activity between cells in both compartments and the extent of functional connectivity between them.

1.6 Use of Co-culture Studies

Primary cell cultures often contain mixtures of different cell types cultured together. Considering the multitude of interconnections that form in unpatterned neuronal cultures, the restraint of neuritic outgrowth to specific regions means a considerable decrease in network complexity. It is useful to develop techniques to deal with the mixtures of cell types in a comprehensive way to understand the network behavior and possibly to manipulate the cell types individually. As cells from different regions of the brain have different native activity states and may also differ quantitatively in their response to environmental cues, a possible solution is to co-culture cells obtained from tissues composed of single dominant cell types or to optimize the culture conditions in order to preserve the cellular identity and discourage proliferation of different types of cells. However, to understand the interaction between neuronal sub-population, we need to develop an environment that can sustain cells from different regions of the brain in isolation while providing proper access to electrophysiological interaction between them. Organotypic slices provide techniques for studying electrophysiological interaction between selected regions of the brain, selective fluidic manipulation of a specific region in an organotypic slice is almost impossible. Hence, developing a co-culture system with dissociated cells in compartmented devices may provide unique manipulation capabilities. To some extend these possibilities complement the modern alternative approach that uses optogenetics to stimulate molecularly defined cell classes (Wen, Wang et al. 2010).
Coronal section view of 18 day embryonic rat brain through cortex and thalamus, with cortex region marked from C1 through C26 and thalamus marked with T1, T3, T27 and T17. The thalamocortical projections here are marked as f8, B13 and B14. Thalamic and Cortex tissues (from the region indicated) were isolated from the rest of the brain region and dissociated cell cultures were prepared from the isolated tissue samples.

THALAMO-CORTICAL SYSTEM AND CO-CULTURE STUDIES

The influence of cortical cues in the development of thalamo-cortical connectivity or in the remodelling of networks following environmental modifications has been demonstrated (Coronas, Durand et al. 2000). Thalamic cells require trophic support from cortex for their survival (Asavaritikrai, Lotto et al. 2003). The functioning of thalamus circuitry and its functional relationship with cortex (Box 4: Schematic view of cortex and thalamus in coronal section view) has been extensively studied both in vivo (Nicolelis 2005; Crunelli, Errington et al. 2011) and in vitro (Adams, Kyi et al. 2011). Recent advancement has set forth new hypotheses questioning the function of thalamus as a mere relay station and if the information that is being passed to the cortex is without significant change in content (Sherman and Guillery 2002). To understand the working of thalamo-cortical sub-circuitry, we must understand the signals that transmit between the two regions (Sherman and Guillery 2002). The mechanisms that have received significant attention in the recent past include sleep and the production of epileptic discharges (Steriade, Nunez et al. 1993; McCormick and Bal 1997). Both in vivo preparations and in vitro slice studies have emphasized that cortical burst firing frequently show rhythmic bursting and large regions of thalamus become synchronized with this rhythmic bursting (Steriade and Deschenes 1984; Steriade and Llinas 1988; McCormick and Bal 1997). However, detection of synchrony in the network usually requires manipulating an individual region and simultaneous recording from both the regions. To this effect, dissociated co-culture system provides means to selectively manipulate the individual region using neuro-active substances and thereby, a possibility to understand the interactions in cortical-thalamic circuitry. This may open new perspectives in the study of connectivity dynamics that are responsible for generating rhythmic neuronal network oscillations in cortical-thalamic system.
1.7 **Selective Chemical Manipulation of Co-Cultures**

Neuronal networks are primary examples of complex systems in which small changes in initial conditions can have a significant effect at network level. Pharmacologically induced changes in network behavior are a reflection of overall effects on pre- and post-synaptic activities, and the morphology of synaptic connectivity in the network (Maeda, Robinson et al. 1995; Nakanishi and Kukita 1998). The actions of neuroactive agents at the network level differ from their actions on individual synapses.

In a co-culture system with two different cell types, manipulating an individual cell type pharmacologically may influence the propagation of signals to the other cell type and can influence the intrinsic behavior of the system depending on the interdependency in network connectivity. In a system involving cortex and thalamus, the role of cortical input in the control of burst and tonic response modes of thalamic neurons is more difficult to assess in vivo.

In this thesis, a system to study the interaction between cortical and thalamic regions in vitro is presented. The system offers only a limited level of network connectivity and environmental conditions as in vivo, it offers sufficient tools to probe the influence of cortical cues on the behavior of thalamic cells and vice versa. The system offers a unique capability to selectively manipulate each of the regions independently and thereby study the interaction in cortical-thalamic circuitry. Development of such in vitro systems utilizing dissociated cells in compartments may offer a complimentary approach to in vivo studies to better understand the interplay between thalamus and cortex.
1.8 Outline Of This Thesis

This thesis describes a dual compartment system for culturing two different cell types interconnected through microchannels. Structural and functional synaptic connections between the two compartments were experimentally verified and selective chemical manipulation of individual cell types was demonstrated using neuroactive substances. In chapter 2, the design and fabrication of the compartmented microfluidic device for neuronal cell culture is presented. To sustain viable cells for long term electrophysiological measurements, partial medium change in the reservoir was facilitated 3 times a week to compensate for the evaporation losses and to provide supplements for the culture (see protocol for medium change in Chapter 2). This medium change frequency offered cell survival thus far resulting in active cultures up to DIV 35.

Cortical-Cortical (Cx-Cx) co-cultures

Dissociated cortical cells were cultured in both the compartments of the device and electrophysiological measurements of spontaneous network activity in the compartments are presented in chapter 3. Biological origin of network activity and the fluidic isolation between the compartments was demonstrated by applying Tetrodotoxin (TTX) in one of the compartments and the corresponding change in electrode spiking in both compartments was measured. Structural connectivity between neuronal populations via the microchannels and the crossing-over of neurites are verified using transfection experiments and immunofluorescent staining. In addition to the neurite cross-over to the adjacent compartment, functional connectivity between cells in both the compartments is verified using cross-correlation based techniques and bidirectional signal propagation between the compartments is demonstrated using functional connectivity maps. Statistical analysis of correlation reveals that the two neuronal populations are not only functionally connected within each compartment but also with each other.

Cortical-Thalamic (Cx-Th) co-cultures

Chapter 4 and 5 discuss culturing heterogeneous co-cultures containing dissociated cortical and thalamic cells in the dual compartment device. Analysis of spontaneous activities from the co-culture showed a different firing pattern for the individual cell types in the culture. Propagation of electrical activities between cortical and thalamic regions and their inter-dependency in connectivity is verified. We propose
that burst events originate in the cortical region and drive the entire cortical-thalamic network bursting behavior while mutually weak thalamic connections play a vital role in sustaining longer burst events in cortical cells.

Selective pharmacological manipulation
The main reason to design the microfluidic system lies in its ability to selectively pharmacologically manipulate the individual compartment of the co-culture. In chapter 6, origin of network wide burst events in the co-culture is validated by selective pharmacological manipulation of individual cell types. Using pharmacological agents such as SRA to selectively control the network-wide burst propagation in Cortical-Cortical and Cortical-Thalamic co-cultures, regions of burst initiation were identified. The results demonstrate that in cortical-thalamic dissociated co-cultures, by controlling network bursts in cortical region, it is possible to control the spread of bursting activity in the thalamic region, in agreement to our earlier findings (reported in chapter 5). However, in cortical-cortical co-culture system, any one of the two regions acts as a site of burst initiation and facilitates propagation of bursts in the entire network. The analysis presented in this chapter is in agreement with the recent findings that cortical region is the site of initiation of burst firing events while reciprocal thalamo-cortical connections are required to maintain a prolonged synchronised bursting pattern in the cortical culture (Adams, Kyi et al. 2011).
Introduction

References


CHAPTER 1


CHAPTER 1

Introduction


Steriade M, Nunez A, Amzica F. A Novel Slow (Less-Than-1 Hz) Oscillation Of Neocortical Neurons


Wagenaar DA, Pine J, Potter SM. An extremely rich repertoire of bursting patterns during the development of cortical cultures. BMC Neuroscience, 7 (11), 2006a.

