Chapter 3

Establishment of a culture model for network activity in neuronal ensembles

3.1 Introduction

As reviewed in section 1.1, neuronal cultures grown on multi electrode arrays have emerged as a successful model for studying generic properties of neuronal ensembles at the network level. Nevertheless, plasticity in these systems has been controversial and accepted induction protocols have not emerged as multiple reports produced contradictory or negative results (reviewed further in section 3.4). This in contrast to *in vivo* systems and slices where activity dependent plasticity induced through stimulation or behavioural paradigms is well established [1]. It should be noted that spike timing dependent plasticity had been demonstrated in hippocampal cultures for pre and post synaptic neuron pairs explicitly controlled and monitored via patch clamp [2]. The lack of success on MEAs cannot, therefore, be attributed so a skewed biology of neurons in culture and it is not yet clear if it is a result of the poorer sensitivity of the extracellular recordings (sensitive only to super-threshold processes) or the culture network topology which needs to be accounted for in the current experimental paradigms.

Given the established contribution of the neuromodulatory system to activity dependent plasticity, adding a neuromodulatory functionality to the culture system would be useful and may enable plasticity. Indeed that dopamine modulates plasticity in culture has been established but using bath application therefore only interrogating tonic effects [3, 4]. Generating rapid neuromodulatory pulses at physiological time scales requires microfluidics technology and is the topic of this Ph.D thesis. Slower pulsing, nevertheless, is possible in traditional *in vitro* experimental systems through manual pipetting of agonist solution followed by re-

placement of the culture media. In this chapter we describe the establishment of a standard cortical neuronal culture (dissected from both rat and mouse embryos) in our laboratory facility. In this work, we followed the development of the cultures over 3 weeks in vitro and demonstrated that they develop normally and exhibit hallmark spiking activity, both spontaneous and evoked. We then revisited the question of plasticity induction and found that a standard tetanization protocol does not produce a measurable change in evoked responses or in functional connectivity in our cultures. Finally, we explored the utility of slow pulsing of dopamine by manually adding dopamine during the plasticity-induction phase (tetanization) and washing it away immediately after. We found that the altered protocol results in a change to activity and connectivity measures but argue that the effect of the washing cannot be easily separated that of the dopamine, hence confusing the interpretation. This chapter thus serves to establish the multi electrode array electrophysiology technique and to provide motivation for the development of the microfluidics technology in the following chapters.

3.2 Spontaneous activity over time in Mouse cultures

images of cultures

example fig with raster plots and the correlation next to it

3.2.1 Activity measures

firing rate figure

3.2.2 Bursting measures

burst figure

3.2.3 Comparison between platings

Refer to previous images with other plating data

3.3 Evoked activity

Evoked activity example in rat culture

3.4 Attempts to induce plasticity

Why I selected the Chiappalone protocol.