# Time Corse, Spatial Distribution, and Patterns of Spontaneous Spiking Activity of Chick Forebrain Neuronal Network Cultured on Microelectrode Array Platform

Yan Kuang[1], Zhonghai Wang[1], Bin Xu[2], Tingfei Xi[3], and Bruce Z. Gao[1]
[1]Department of Bioengineering, Clemson University, Clemson, SC
[2]National Engineering Laboratory for Regenerative Medical Implantable Devices, Guangzhou, China
[3]Center for Biomedical Materials and Tissue Engineering, Peking University, Beijing, China

**Statement of Purpose:** There is an urgent need to develop rapid screening-based biosensors to assess the potential neurotoxic effects of large quantity of environmental chemicals. Using microelectrode array (MEA) technology to assess how the spontaneous network spiking activity (SSA) of neuronal culture changes in response to the insults from neurotoxins has become a current trend (Johnstone, et. al., 2010). However, a vast majority of MEA experiments were done using dissociated cortical or hippocampal neurons from rats and mice that are costly. Since the basic neurophysiological features (excitability, conductivity, synaptic transmission, and synaptic plasticity etc.) as wells as the types of neurotransmitters are commonly shared by both avian and mammalian, it is possible to develop neurotoxin biosensors using chick neurons. However, since chick neuron cultures have a short life span about two weeks, a potential concern is whether during its life span, the cultured chick neuronal network has enough time to develop relatively stable SSA that allows the neurotoxin assay? To answer this question, two specific aims need to be achieved in this study: 1) to prolong the life span of chick neurons in culture by all means; 2) to characterize the SSA signals recorded with MEA from chick neuron cultures.

**Methods:** E8 chick forebrain neurons (FBN) were dissected, dissociated, and plated on a sterilized MEA (200/30iR-Ti-gr, ALA Scientific) pre-coated with Polyethylenimine (PEI). To prolong the life span of FBN in culture, we plated cells at a high density of 3000 cells/mm$^2$ without any treatment to inhibit glial growth. Medium 199 ( 11043, Gibco) supplemented with 10% FBS and 2% B27 were used. The MEA was covered with a Teflon® lid (ALAMEA-MEM5, ALA Scientific), kept in a 100-mm Petri dish, and placed in a dry incubator at 37 °C and 5% CO2. The Teflon® lid, permeable to gases but not water and bacteria, prevented the culture on MEAs from loss of humidity due to evaporation and contamination (Potter, 2001). A dry incubator allowed MEA recording to take place inside the incubator. Media were half changed once or twice a week. To obtain the time course, spatial distribution, and the patterns of the SSA from the developing neuronal network in culture, cultures on MEAs were measured 10 min daily from day 2 through their whole life span. Based on these results, the life stages of the cultured neuronal networks (i.e., developing, maturing, and degenerating) were characterized. A rapid increase in the rate of SSA and a subsequent plateau for a few days were expected to be supportive to the feasibility of developing MEA-based chick neuronal biosensor.

**Results:** 1) When plated at high density of 3000 cells/mm$^2$ without inhibition of glial growth, the life span of the chick FBN cultures could be increased from about 14 days to around 18 days. 2) Sporadic single spikes could be detected since 4 days in vitro (DIV) within our 10-min time window of MEA recording, but net-wide spiking synchronization occurred as early as 5 DIV, suggesting a very rapid process of synaptogenesis in comparison with a relatively slow steady increase seen in rat neuronal cultures. 3) As long as SSA occurs, it kept robust firing through its whole life span. 4) As shown in Figure 1, From 5 DIV to 9 DIV, the active channels that showed SSA among a total of 59 recording channels corresponding to 59 microelectrodes on the MEA surface increased rapidly, and reach its maximum at 9 DIV that is about 86% of the total channels. Two out of seven MEA traced cultures remained at this maximum active channel distribution for more than five days, while others showed a reduction in the active channels gradually. More experiments are needed to figure out how to keep the maximum spatial firing distribution at a plateau for more days. 5) The patterns of the SSA include spikes, bursts, and synchronized spikes and bursts that are as typical as those seen in rat cortical networks, but the time course is much shorter.

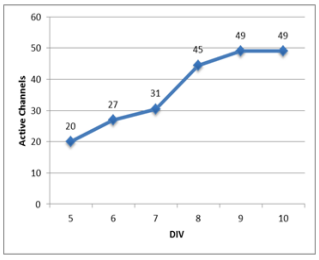

Fig 1. Active channels indicating the spatial distribution of SSA increases by days in vitro.

**Conclusions:** 1) It is possible to prolong the life span of chick FBN cultures. 2) The patterns of network firing activities of chick FBN are quite similar to those seen in rat cortical cultures. 3) The very early occurrence of the SSA synchronization and the rapid increase in active channels suggest a very rapid development of the network with maturing synapses. 4) These data are supportive to the feasibility of developing MEA-based chick neuronal biosensors.

**References:**

Betts KS. Environmental Health Perspectives. 2010; 118: A432-A437
Johnstone AFM. NeuroToxicology. 2010; 31: 331-350
Potter SM. Journal of Neuroscience Methods. 2001, 110: 17–24