LS100r- Fall 2013
Project 1

Examining the Influence of Precise Neural Activity Patterns on the Development and Differentiation of Human Neural Precursor Cells.

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Background

In the mammalian brain, the firing patterns of neurons are know to play an essential role in cellular and network development; however, exactly how neural activity affects cell differentiation and connectivity is not understood. This question is significant for two major reasons. First, scientists are now beginning to generate faithful (and individual-specific) disease models that are experimentally tractable from human precursor/stem cells. To do so, we need to guide neural differentiation to specific cell types. This process likely involves regulating and modulating neural activity. Second, the rules that govern how developmental synaptic connectivity is influenced by activity have not been tested, and they are essential to understand how the brain gets 'wired up'. With our model system, we will explore both questions.

Until recently, there has been no way to precisely manipulate activity *in vitro* during a long (multi-day) period of development and induced differentiation. Technological breakthroughs have lead to light-sensitive cation channels that can be stimulated with millisecond scale light pulses to mimic neural activity non-invasively.

In this project, we will first characterize a new model system: human fetal neural precursors taken from the fetal ventral midbrain (ie, dopaminergic areas) to monitor the development of voltage-gated channels and other key neurophysiological indicators during typical differentiation. Next, we will transduce a light-sensitive channel (Channel Rhodopsin) into the cells to manipulate the electrical firing pattern of the neurons with LED lights in culture. Finally, we will examine how the different patterns of activity we induce affect cell type specification, survival, and connectivity using confocal microscopy.

Goals & Approach

The goals of the project are:

1. Learn to use and understand patch-clamp and whole-cell recording techniques to examine the development of electrophysiological properties of *in vitro* differentiated neurons.

2. Learn to use and understand extracellular recording techniques and LED stimulation to develop a novel in human neural precursor model with Channel-Rhodopsin expression.
3. Create a method for stimulating neurons during *in vitro* differentiation with precise temporal patterns of light to mimic *in vivo* electrical activity.
4. Use immunocytochemistry and confocal microscopy to characterize connectivity among neurons with coordinated firing patterns.

To accomplish these goals, we will learn about cellular neurophysiology (ie, how neurons act as electrical units), activity-dependent neural development (ie, how patterns of action potentials affect cell type development and connectivity), and classic and cutting-edge methods specific to neurobiology (electrophysiology, optogentics, and fluorescence microscopy).

Background Reading

Li I. Zhang & Mu-ming Poo. **Electrical activity and development of neural circuits.** *Nature Neuroscience* **4**, 1207 - 1214 (2001).

Boyden, E. S., Zhang, F., Bamberg, E., Nagel, G., and Deisseroth, K. (2005) **Millisecond-timescale, genetically-targeted optical control of neural activity.** Nature Neuroscience 8(9):1263-1268.

Roberta Donato, Erik A Miljan, Susan J Hines, Sihem Aouabdi, Kenneth Pollock, Sara Patel, Frances A Edwards, and John D Sinden. **Differential development of neuronal physiological responsiveness in two human neural stem cell lines.** BMC Neurosci. 2007; 8: 36.

Natalia V. De Marco García, Theofanis Karayannis & Gord Fishell. **Neuronal activity is required for the development of specific cortical interneuron subtypes.** Nature 472, 351–355(21 April 2011).