Application Note: LC01682000

A High Content Screening Approach for Measuring Neuronal Function

Amy Peters, Donna Miller, Gang Lin, and Jeffrey Haskins
Thermo Fisher Scientific, Laboratory Automation and Cellular Imaging · 100 Technology Dr., Pittsburgh, PA 15219

Key Words

- Pathophysiological
- Morphology
- Neurons
- Neurites
- Cell bodies

Introduction

Neurotrophins act to control developmental cell death of neurons. However, neuronal abnormalities (neurodegeneration, neurotransmitter imbalance, etc) can be implicated in a variety of pathophysiological conditions such as with Alzheimer's, Parkinson's, and psychiatric disorders. These disorders affect changes in neuronal cell morphology and/or changes in neurotransmitter release. Some disease conditions affect outgrowth and elongation of neurites, branching of the neurites, changes in neuronal cell survival, cell body area, and expression of certain genes.

Synapses allow neurons to communicate with each other via the release of a neurotransmitter that opens ion channels or activates second messenger systems. Overall, synapse formation is a complex process that requires appropriate projection of the axon to a specific target followed by the synthesis and targeting of multiple ion channels proteins to the synaptic site. Pre-synaptic cells will have neurotransmitters that accumulate in small vesicles, with post-synaptic sites exhibiting clustering of receptors to receive neurotransmitters released from the pre-synaptic vesicles. Understanding the purpose of these targets and their relationship to overall neuronal functioning can help discover the meaning and possible treatment of neurological disorders.

The objective of this study was to characterize neurons by evaluating cell body and neurite morphological differences (through cell body shape, neurite length, neurite width, and branching pattern features), as well as looking at neurite functional characteristics. Our data confirms that a high content analysis approach can accurately characterize neuronal cultures with respect to their morphological characteristics as well as neuronal function and can become a valuable way to conduct in-depth classification of this biology.

Materials and Methods

Cell Culture Rat brain hippocampal cells were cultured according to the supplier's instructions (Lonza, Allendale, NJ) and plated at approximately 9000 cells/well in poly-D-lysine/Laminin 96-well plates (BD Biosciences, Franklin Lakes, NJ). Cells were incubated at 37ºC, 5.0% CO_2. After 4 days *in vitro* various serially diluted compounds or recombinant proteins were added to culture media. Compounds and/or media replacement occurred every three to four days during the 14 day course.

Immunocytochemistry

After 14 days of treatment, cells were fixed with a pre-warmed paraformaldehyde solution (3% final concentration) for 20 minutes. Plates were washed twice with and then incubated in Neurite Outgrowth (NOG) Buffer (Thermo Fisher Scientific, Rockford IL) for 30 minutes. Antibodies to ß3 Tubulin and Synaptophysin-1 diluted in NOG buffer were incubated for 90 minutes. Plates were then washed twice with NOG buffer and the appropriate secondary antibodies were incubated for 60 minutes. After the final incubation, plates were washed twice with NOG buffer and twice with PBS. Plates were imaged with the Thermo Scientific Cellomics® ArrayScan® V^TI HCS Reader and simultaneously analyzed with the Neuronal Profiling v3.5 BioApplication.

Thermo Scientific Cellomics Neuronal Profiling v3.5 BioApplication

The Neuronal Profiling v3.5 BioApplication quantifies morphological changes in neurons. It allows the user control over selecting neurites based on differences in morphology and intensity, as well as selecting neurons based on parameters in the nuclear and neuronal channels (Figure 1). Additionally, the user can identify spots found within/along the neurites or cell bodies and can be used to identify spot overlap between two channels.

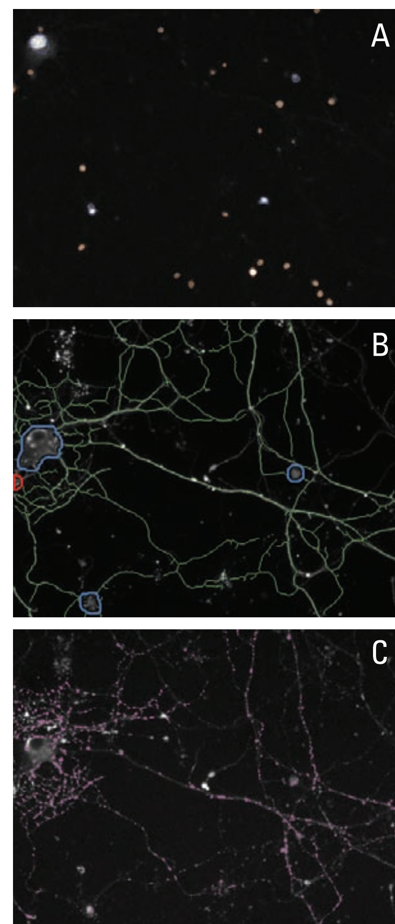

FIGURE 1. Processed images identifying nuclei that were stained with Hoechst (A; blue), and indirect immunofluorescence against neuronal cells (B; cell body, cyan; neurite, green), and pre-synaptic spots (C; cell, pink).

Results

Neuronal Morphological Characteristics

Comparisons were made in rat primary hippocampal neurons with four different compounds dosed in duplicate and grown for a total 14 days *in vitro* (DIV).

Morphological features such as neuronal area, neurite count and length, and number of branch points are shown. All data was compared as a percent of averaged control wells (untreated cells).

At 14 DIV (Figure 2): cell body area measurements varied between compound and dose. SU6656 and Bis-1 had decreased neurite count, neurite length and branch point count below control values (2.2 - 20 µM SU6656 and 1.3 - 12 µM Bis-1). Thyroxine was similar to control values and Dopamine was slightly above control values (~120%) at 333 nM.

Synaptic function results

Pre-synaptic functionality measured using a synaptophysin specific antibody in both cell body and neurites were observed. Synaptophysin-1 spots (Figure 3) found in the cellbody were below control values for Bis-1 until 1.3 µM. However, overall cell body spot area was greatly above control for Bis-1 and SU6656 at higher doses. Higher values of thyroxine caused decreased cell body spot area but increased neurite spot count and area.

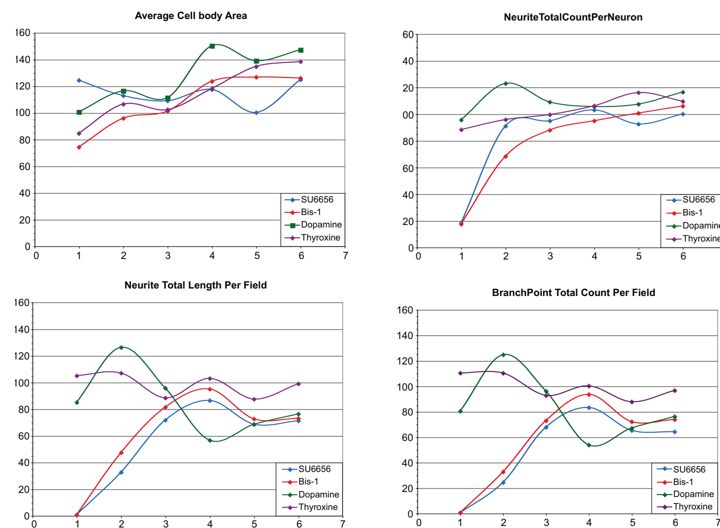

FIGURE 2. Effects of various compounds after 14DIV on (top left) average cell body area, (top right) neurite total count per neuron, (bottom left) neurite total length per field, and (bottom right) branch point total count per field. X-Axis represents dilutions (1 = highest dose) and Y-axis represents % of averaged control.

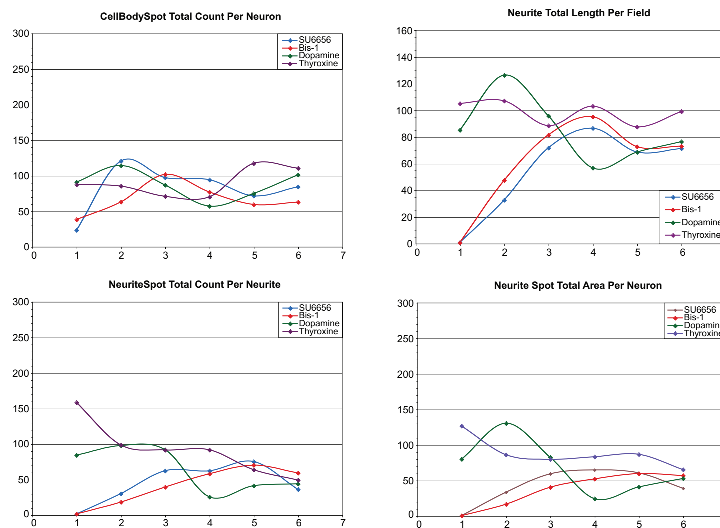

FIGURE 3. Synaptophysin differences in cell body and neurites when comparing compounds after 14DIV as (top left) cell body spot total count per neuron, (top right) cellbody spot total area per field, (bottom left) neurite spot total count per neurite, and (bottom right) neurite spot total area per neuron. X-Axis represents dilutions (1 = highest dose) and Y-axis represents % of averaged control.

Conclusions

- Both outgrowth inhibitors SU6656 and Bis-1 had significant effects at the highest concentration tested but did not have a true dose-dependent effect on overall neurite length. This could be due to having cells cultured for four days before treatment.
- Dopamine and Thyroxine are important in overall neuronal function. Therefore, it was expected for overall neurite spot count to be at or above control values. As concentrations decreased so did the number of neurite spots.
- The Thermo Scientific Cellomics Neuronal Profiling v3.5 BioApplication is a functional tool to identify neuronal cells, trace neurites of various lengths, and multiplex both neuronal and functional characteristics in primary neuronal cultures.

References

1. Thoenen, H. 1991. Trends Neurosc. 14:165-170.
2. Ooyen, A.V., J.V. Pelt, and M.A. Corner. 1995. J. Theor. Biol. 172: 63-82.
3. Fields, R.D. and P.G. Nelson. 1992. Int. Rev. Neurobiol. 34:133-214.
4. Meriane, M., J. Tcherkezian. C.A. Webber, E.I. Danek, I. Triki, S. McFarlane, E. Bloch-Gallego, and N. Lamarche-Vane. 2004. J. Cell. Biol. 167: 687-698.
5. Paveliev, M., M.S. Airaksineen, and M. Saarma. 2004. Mol. Cell. Neurosci. 25:453-459.
6. Perez-Delgado, M.D., R. Ferres-Torres, A. Castaneyra-Perdomo, and T. Gonzalez-Hernandez. 1987. J. Anat. 150: 23-29.

In addition to these offices, Thermo Fisher Scientific maintains a network of representative organizations throughout the world.

Laboratory Automation and Cellular Imaging
100 Technology Drive
Pittsburgh, PA 15219

U.S.A.
Toll Free:
1.800.432.4091
Sales:
412.770.2200
Fax:
412.770.2450

Europe:
Sales:
+44(0) 118.988.0262
Fax:
+44(0) 118.988.0362

Japan:
Sales:
+81-3-5684-6199
Fax:
+81-3-5456-5511

For more information visit:
www.thermo.com/cellomics

©2007 Thermo Fisher Scientific Inc. All rights reserved. All trademarks are the property of Thermo Fisher Scientific Inc. and its subsidiaries.

AP-LACI-NP3.5-1107