High-Content Analysis
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High-Content Analysis of Primary Rat Neural Cortical Cultures for Developmental Neurotoxicity Screening Applications

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Development of the vertebrate nervous system proceeds through a number of critical processes, ultimately concluding with the extension of neurites and establishment of synaptic networks. Early-life exposure to toxicants that perturb these critical developmental processes can potentially result in deficits in CNS function in later life. Efficient methods for identification and characterization of potential developmental neurotoxicants (DNT) are needed to support regulatory efforts. The present work describes the development and implementation of high-content image analysis (HCA) assays for screening chemical effects on neurite outgrowth and synaptogenesis in dissociated primary mixed neural cultures from new-born rat neocortices. For both endpoints, dissociated cortical cells were seeded at densities ranging from 20,000 to 40,000 cells / well (6.7x104 to 1.3x105 cells / cm2) in poly-L-lysine coated 96-well culture plates. The time course for neurite outgrowth was examined throughout the first 120 h of growth and the time course for synapse formation examined from 6 days in vitro (DIV6) to DIV19. The concentration-response for a variety of compounds known to inhibit neurite outgrowth and synapse formation were examined in parallel with measurements of cell viability (ATP content, CellTiterGlo®). For each endpoint cells were allowed to develop for the appropriate time period and then fixed in the presence of Hoechst 33342 (nucleus label), sequentially immunolabeled with antibodies targeted against β-III-tubulin (neurite outgrowth) or MAP2 & synapsin (synaptogenesis) and DyLight™-conjugted fluorescent secondary antibodies. Cells were then imaged using a Cellomics VTI HCS Reader and analyzed using the Cellomics Neural Profiling BioApplication. The average number of neurites and total neurite length per neuron increased dramatically in cortical cultures between 2 and 120 h. Cultures exposed to either bisindolylmaleimide I (0.1 – 10 μM), Na3VO4 (1 – 100 μM) or LiCl (0.3 – 30 mM) for 2 to 24 h post-seeding had concentration-dependent decreases in neurite outgrowth. For synaptogenesis, the total area of synapsin staining associated with neuronal cell bodies and dendrite-specific MAP2 staining increased between DIV9 and DIV15. Concentration-dependent decreases in the area of synapsin staining was observed in cultures exposed to the cholesterol-synthesis inhibitor mevastatin (0.3 – 30 μM). These experiments demonstrate that primary mixed cortical cultures can be used to examine chemical effects on neurite outgrowth and synaptogenesis and may be appropriate for using as a DNT screening tool. This abstract does not necessarily reflect USEPA policy.