A HIGH CONTENT IN VITRO SCREEN FOR MEASURING REGULATION OF SYNAPSES BY GENETIC AND PHARMACOLOGICAL TARGET MODULATION

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1. A comprehensive set of in vitro and in vivo tools

The Evotec platform studies CNS diseases by modulating risk gene expression using Aden-Associated Virus (AAV) mediated gene transfer or knock-down. On the one hand an in vitro neuronal culture system can be used for phenotypic assays or compound profiling. On the other hand in vivo intervention with specific signaling pathways can be tested using AAVs targeted to specific brain regions via stereotaxic injections. Both of these approaches use high content analysis to measure effects at the synaptic level. This enables identification and validation of disease relevant targets and in the case of in vitro models provides a platform for small molecule screening.

2. Automated synapse detection

The assay we have developed measures the co-localization of pre- and post-synaptic markers and is used to quantify the density of synapses. It can also be used to measure the effect of genetic or pharmacological modulation of targets at the synaptic level. Automated image analysis of pre- and post-synaptic markers was performed on primary cultures of hippocampal neurons using in-house developed Acapella®-based scripts (Figure 3B). To determine the sensitivity of the assay, synaptic transmission was pharmacologically enhanced using picrotoxin (PTX) for a period of 3 days. This treatment produced a reduction in the density of synapses (Figure 3C). The effect of PTX could be reversed by inhibiting synaptic connectivity using tetrodotoxin (TTX) and also by a selective block of NR2B-subunit-containing NMDA receptors by dextropropoxyphene (Figure 3C).

3. Spine identification using Evotec developed analysis scripts and Acapella workflow

To complement the synapse density measurements described above, we also developed a reproducible technique for visualization of dendritic spine size and morphology by GFP® or RFP® transfection (Figure 4). Individual neurons within each culture are labelled and are suitable for automated high content measurements of spine morphology using Evotec developed Acapella® scripts. This method is suitable for pharmacological intervention protocols but also enables to analyze the direct effects of AAV-mediated gene over-expression or knock-down on spine density, spine size, and morphology.

4. In vitro modeling of synaptopathies for phenotypic screening

Disruption of synaptic structure and function is thought to be a major determinant of neuro-degenerative and psychiatric disorders. One example is Autism spectrum disorder (ASD); primarily characterized by behavioral and social impairments. Numerous genes have been linked to this disorder, many of which are associated with synaptic dysfunction.

Conclusion

Evotec have developed multiple assays to induce and measure changes in synaptic structure and function in primary cultures of rat and mouse hippocampal neurons.

- Used the Opera® platform and high content image analysis to detect co-localization of pre- and post-synaptic sites in 96- and 384-well imaging plates
- Developed a reproducible technique for GFP® labeling of single neurons in primary cultures to visualize dendritic spines in 3D
- Used live Ca2+ imaging at the FLIPR to assess the neuronal network activity of the culture (data not shown)

Modulated risk gene expression by viral transduction to derive translational models for ASD

This platform can be used to identify/validate targets and/or small molecules capable of regulating neuronal synapses. Such information will be integral for the development of CNS disease therapies.

References


Evotec has developed a drug discovery platform which uses state-of-the-art high content imaging technology which can be used for in vitro and in vivo analysis of synapse number and structure. This platform can be used for target validation studies and also for compound profiling.

This builds on about 20 years of CNS and drug discovery experiences at Evotec and combines Opera®-based imaging with sophisticated analysis tools.

**Introduction**

- Synaptic dysfunction is a common early event in neuro-degenerative diseases and age-associated cognitive decline.
- Monitoring synaptic integrity in cultured neurons could serve as readout system for a variety of memory disorders (Figure 1).
- Importantly, loss of synaptic connectivity may prove to be a reversible phenomenon suitable for drug intervention. In contrast, neuronal loss occurring at disease end-stage is unlikely to be readily reversible. Indeed current therapeutics for Alzheimer’s disease, the most prevalent cognitive disorder in the world today, focus on enhancing the signaling properties of remaining neuronal population rather than addressing the disease progression itself.
- Despite a clear link between spines, synaptic connectivity, neuro-degenerative and neuro-developmental diseases there remains a distinct lack of tools to assay such processes within modern drug discovery workflows.

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