MODULATION OF SYNAPTIC STRUCTURE AND FUNCTION IN PRIMARY RODENT HIPPOCAMPAL NEURONS BY AUTISM RISK GENES-A HIGH CONTENT IMAGE ANALYSIS

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Background
- Synaptic dysfunction is a common early event in neuro-degenerative diseases and age-associated cognitive decline.
- Despite a clear link between synaptic spines, synaptic connectivity, neuro-degeneration and neuro-developmental diseases there remains a distinct lack of tools to assay such processes within modern drug discovery workflows.
- Monitoring synaptic integrity in cultured neurons could serve as readout system for a variety of mental disorders (Figure 1).
- Evotec has developed a drug discovery platform which uses state-of-the-art high content imaging technology for in vitro and ex vivo analysis of synapse number and structure. This platform can be used for target validation studies and also for compound profiling.

1. Automated synapse counting using Evotec developed analysis scripts

Automated image analysis of pre- and post-synaptic markers was performed on primary cultures of hippocampal neurons using in-house developed AcapellaTM-based scripts (Figure 2A–B). To determine the sensitivity of the assay, synaptic transmission was pharmacologically enhanced using forskolin (PTX) for a period of 3 days. This treatment produced a reduction in the density of synapses (Figure 2C). The effect of PTX could be reversed by inhibiting synaptic connectivity using tetrodotoxin (TTX) and also by selective block of NMDA-signal-containing NMDA receptors by kynurenic acid (KYN) (Figure 2D).

To model diseases at a synaptic level we have used AAV-mediated shRNA to knock-down key Autism Spectrum Disorder (ASD) related genes. The resulting "synaptophagy" may be suitable for in vitro phenotypic rescue experiments using small molecule libraries. Preliminary data indicates gene-dependent up- and down-regulation of synapse density (Figure 2).

Conclusion
Evotec has developed multiple assays to induce and measure changes in synaptic structure and function in primary cultures of rat and mouse hippocampal neurons.
- Used the OpenRail platform and high content image analysis to detect colocalisation of pre- and post-synaptic sites in 96- and 384-well imaging plates
- Developed a reproducibility technique for GFP labelling of single neurons in primary cultures to visualize dendritic spines in 3D
- Used electrophysiological measurements to assess how structural or density changes alter functional connectivity (data not shown)

Modulated risk gene expression by viral transduction in order to derive translational models for ASD
- It is suggested that this platform can be used to identify/validate targets and/or small molecules capable of regulating neuronal synapses. Such information would be beneficial for the development of CNS disease therapies.

2. Spine identification using Acapella workflow

Confocal stacks of shFP-transfected hippocampal neurons were imaged using an OpenRail High-Content Screening System. A total of 30 dendritic regions were subjected to analysis using Acapella automated scripts and compared to Imaris Biplanew® workflows.
- Using the current workflows the Imaris method detected more objects as spines. These tended to be more spread out in shape (Figure 4A-C). These lapses were defined as pin (Vo<0.25m³), L-0.8mum or thin (Vo<0.25m³, L<0.8mum) depending on their length.
- Total number of mushroom spines (Vo<0.25m³, L>0.8mum) and stubby spines were approximately the same using the two methods (Figure 4A-B).
- When tested against a target known to alter spine stability, the Acapella automated detection method was able to detect a significant increase in spine volume after shRNA-targeted gene knockdown.

References