In vitro screening tools to assess effects of psychedelic substances on structural and functional neuroplasticity in rat primary neurons

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BACKGROUND

Some psychedelic drugs have shown promise as therapies for treatment-resistant depression and post-traumatic stress disorders, although underlying mechanisms are not fully understood. Beneficial effects of psychedelic substances, like psilocin or ketamine, on neurons, especially neurite outgrowth, were recently described in independent publications. In most of these publications Scholl analysis or other semi-high-throughput analysis approaches were used to determine structural and functional plasticity of *in vitro* neurite networks. To efficiently screen for therapeutic effects of similar substances or developmental compounds, high-throughput platforms need to be optimized for this specific group of substances.

MATERIALS and METHODS

For that purpose, primary cortical neurons derived from E18 Sprague Dawley rat embryos were isolated and treated with different psychedelics, including psilocin, 2,5-Dimethoxy-4-iodoamphetamine (DOI) and ketamine. Different treatment schedules, treatment durations, time points of treatment, and cell densities were tested for their effectiveness. Analysis of time-resolved neurite outgrowth, as well as spontaneous activity in automated high-throughput applications on Sartorius IncuCyte® Live-Cell Analysis System was performed.

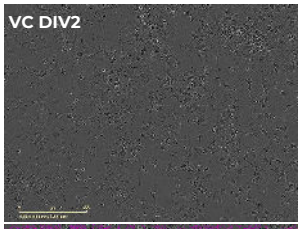
SUMMARY and CONCLUSION

Contrary to Scholl analyses, where the focus lies on individual cells, automated assessment of neurite outgrowth and of the entire cell population within one well was performed. Automated assessment of all treated neurons led to only small effective windows when compared to the respective vehicle controls (Figure 1). Adjustment of cell density or treatment duration/time point did not positively impact the treatment window observed in neurite outgrowth and branching assays (only 20.000 cells/well and DIV1-4 treatment data are shown, Figure 1).

Focusing on activity-based read-outs, including indirect analysis of spontaneous activity by measuring calcium oscillation, revealed significant beneficial effects on network activity and synchronization, but only within a short time frame (Figure 3).

The effect of psychedelic substances on neurons *in vitro* depends on various factors, particularly timing of treatment and analysis are of relevance. High-throughput platforms thus need to be optimized for this specific group of substances to obtain significant treatment windows.

RESULTS



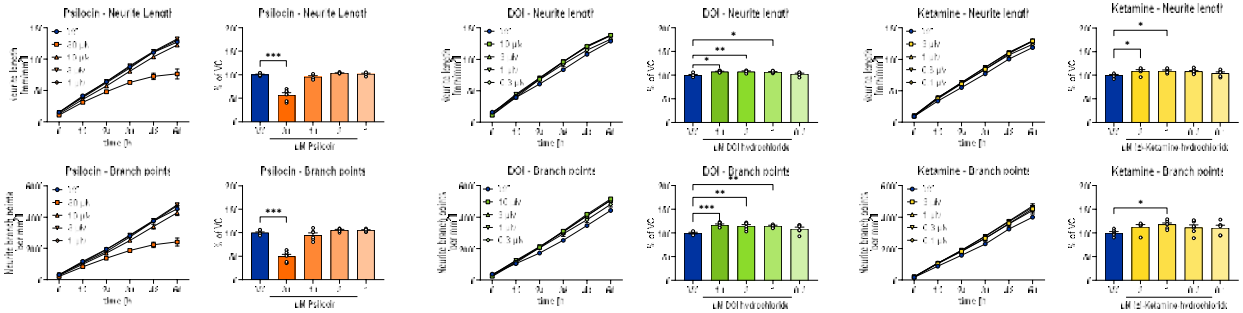


Figure 1 ▲. Effect of Psilocin, DOI and Ketamine on neurite outgrowth and branching assessed by the IncuCyte® Live-Cell Analysis System in cortical rat neurons. Cortical rat neurons were treated on DIV1 with respective items or vehicle control (VC) and maintained in treatment medium until DIV4. Neurite length (top row) and Branch points (bottom row) were evaluated over time (kinetic curve) and after 60 h treatment (bar graphs). Data are displayed as % of vehicle control (VC) and presented as mean + SEM (n=6 per group). For statistical analysis of bar graphs, One-Way ANOVA followed by Dunnett's multiple comparison test (post-hoc test) compared to VC was used. *p<0.05; **p<0.01; ***p<0.001.

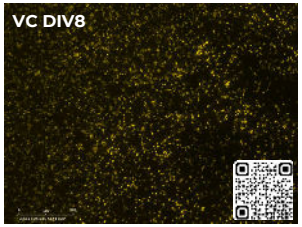


Figure 2 ▲. Visualization of spontaneous neuronal activity via genetically encoded calcium indicator (GECI). Representative image/video (QR code) of vehicle treated primary rat neurons on DIV8 (VC DIV8), as well as representative intensity traces of all active objects within vehicle treated primary neurons on DIV5 and DIV8. Every colored trace in VC DIV5 and VC DIV8 represents activity of a single cell monitored over 180 sec. Traces represent the maturation of the neuronal network, seen as higher synchronicity and stronger bursting.

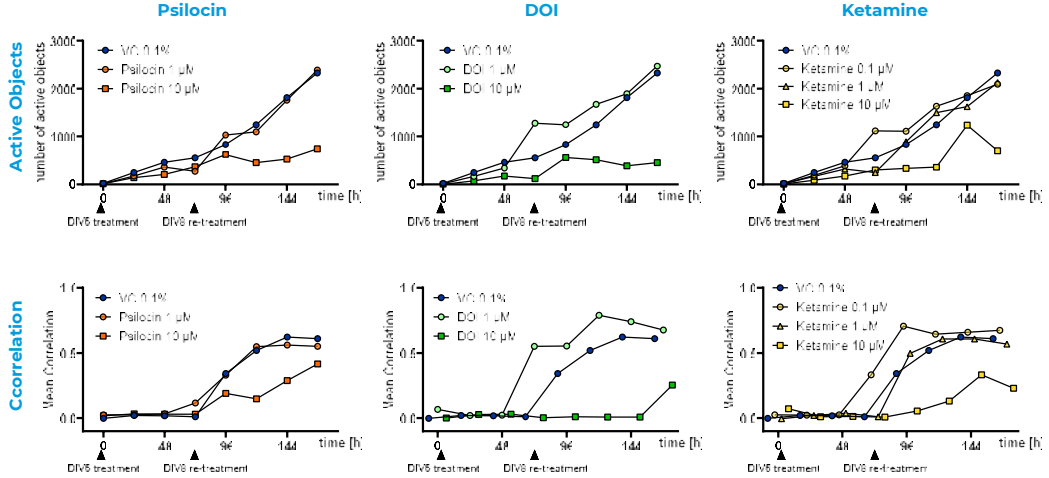


Figure 3 ◄. Monitoring neuronal activity in response to Psilocin, DOI and Ketamine treatment. Cortical rat neurons were transduced with GECI on DIV3 and on DIV5 treatment with respective items or vehicle control (VC) as well as monitoring of cells started. On DIV8, a re-treat in course of a half media change was performed. Number of active neurons per well (objects that burst at least once per recording time; top row) as well as correlation of neuronal activity (as mean of all objects in relation to each other, 0 being completely random and 1 being highly synchronized; bottom row) were assessed using the IncuCyte®.